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JUNE, 1950

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Sognnaes (p. 260) has made excellent preparations of the enamel lamellae and shows that they are not poorly calcified enamel rods. He observed lamellae frequently in erupted teeth but found them extremely rare in unerupted teeth, suggesting that they are not developmental in origin. Sognnaes proposes that the organic matter is in cracks produced by trauma and that it represents a crude form of repair. Since these cracks appear to be the lamellae, the lamellae may serve a useful, not a detrimental, role. Sognnaes's findings are most valuable and may serve to explain the long time split between oral histologists who thought all lamellae to be cracks and those who thought most cracks to be lamellae. It appears that lamellae and cracks are one and the same. The distribution of these organic structures on surfaces not prone to caries is significant.

Walsh and Green (p. 270) have investigated, in vitro, the possibility of protecting tooth surfaces against decalcification by use of long chain paraffin films. The possibility of such a protective action was suggested by reduced caries activity in animals fed diets with supplementary fats and oils. The trick is to bind the fatty material to the tooth surface. Walsh and Green's study suggests that cetylamine may form a bond between paraffin and the enamel surface, resulting in a relatively stable surface film. Clinical studies of this approach are already underway.

Yardeni (p. 278) has contributed the results of his work which was interrupted by war in Israel. He subjected whole teeth and powdered enamel, dentin, and apatite to varying pH levels in different buffers and measured weight loss. The attack on the enamel of intact teeth was at first slow, a lag period being present. Yardeni interprets this as representing the time required in overcoming a biological barrier probably due to the organic matrix. He suggests the possible importance of this period as allowing time for salivary buffers to compensate sudden pH drops, thus protecting the teeth. Various roles have been proposed for the organic material as a passive resistance to dental caries. In opposition to this, there are those who propose that the organic material is the substance in which caries is initiated. At the present time the overwhelming weight of evidence appears to favor the organic material in a mildly defensive role.

Besic (p. 285) has investigated the possible diffusion of solutions applied to teeth. He used steel balls and corrosive solutions to study diffusion in regions comparable to contact points and capillary tubes to simulate pits and fissures. The results suggest the difficulty of reaching the susceptible regions with anticaries solutions. Besic introduces one question that has been in the minds of many investigators, that of the relative effectiveness of fluorides in each of the classes of lesions. If the breakdown of the results of past studies are not made available, new studies should be designed so that the relative effectiveness of fluorides against caries in different teeth and in different types of lesions may be obtained.

Martin and Hill (p. 291) compared the fluorine content of the saliva, salivary lactobacillus counts, and the prevalence of dental caries in Evanston children after two years of exposure to artificially fluorinated water. There was less fluorine in the saliva of 12-, 13-, and 14-year-olds (0.20 ppm) than in 6-, 7-, and 8-year-olds (0.25 ppm). No relation was found between salivary fluorine and oral lactobacilli counts or dental caries prevalence. No relationship was demonstrated between past caries experience and lactobacillus counts. Continued studies from the areas of artificial fluorination are important but we must always bear in mind that results found now may or may not have been influenced by the fluorination. The failure to find a correlation between the number of cavities present and the lactobacillus counts has been interpreted by some as an indication of inadequacy of the bacterial count. Rather one should be considered as an historical record (like the pox mark of smallpox), the other as a test of susceptibility (like the Dick test).

Snyder and Clarke (p. 298) compared the positive color reactions of saliva specimens incubated in bromeresol green dextrose agar with the lactobacillus counts of portions of the same salivas. They point out that the color reaction test lends itself more readily to use by the dental practitioner who should make daily readings for three days after inoculation unless a positive color change is obtained earlier. The Snyder

test appears to be well established in the relationship to the salivary lactobacillus count, but direct correlations with clinical caries have not been documented. However, the test offers a much simpler method for mass screening of individuals for caries susceptibility as well as a usable clinical bacteriologic test.

Douglas (p. 304) points out the numerically significant occurrence of *M. lactilyticus* in all salivas examined. It is most interesting that the organism can ferment only lactate, pyruvate, and malate and its presence probably would depend on the conversion of sugars to lactic acid by other oral inhabitants. Since *M. lactilyticus* converts lactic acid to a weaker acid (propionic or acetic) its significance in the carious process should be carefully considered.

Dreizen, Greene, and Spies (p. 307) added each of 17 cereal products to culture media inoculated with oral lactobacilli. They found that external sources of amylase and of certain members of the vitamin B group were necessary for maximum utilization of cereal products by lactobacilli in vitro. Utilization of the cereal depended largely upon the quantity and type of starch. In general, the more refined the cereal the greater amount of acid produced. It then appears that not only utilizable carbohydrate and acidogens must be present for acid production, but also amylase, thiamin, and nicotinic acid (or substitutes). The absence of these latter factors may explain some apparently paradoxical instances of absence of acid production in the presence of reduced carbohydrates and acidogens. Perhaps even more important factors await discovery.

McClure (p. 315) found that there was no variation in the ash, calcium, and phosphorus content of human enamel with either age or caries experience. An eight times increase in enamel fluorine content of enamel did not change the ash, calcium, or phosphorus content, nor did a tenfold increase in dentin fluorine effect change the calcium or phosphorus content. A slight increase in the ash content of highly fluorinated dentin was observed. The author interprets his data to indicate that fluorosed enamel is not due to any reduction in calcification. How this is to be considered in view of the pathologic and histologic interpretation of mottled enamel as a form of enamel hypocalcification is not discussed. Is mottled enamel not a form of hypocalcification? Are the methods of chemical analysis too gross to show the changes? Chemical analyses of nonfluorinated hypocalcified enamel might give some useful data in this regard.

From an in vitro study, Kirchheimer and Douglas (p. 320) were unable to demonstrate inhibiting effects of 0.25 to 0.5 per cent concentrations of ammonium salts against eight strains of lactobacilli. These findings, while not confirmed in vivo, suggest that if dentifrices or chewing gum containing small quantities of ammonium ion inhibit dental caries, the mechanism probably is not due to the effect of the ammonium ions, per se, on acidogenic organisms.

Brucker (p. 325), investigating the incidence of fissures and caries found almost equal occurrence of fissures in the first and second deciduous molars, although caries is more common in the second. He could find no substantiation for the theory that fissures and caries occurrence are related in the deciduous teeth.

Baume (p. 331), studying the development of the occlusion of 60 children by serial plaster reproductions of the dental arches, concluded that the first permanent molars erupt directly into proper occlusion without altering the position of the neighboring teeth as a result of the occurrence of a terminal plane in the deciduous denture. The presence of a previously described mandibular primate space and the straight terminal plane allow a proper molar occlusion by means of an early mesial shift of the mandibular deciduous molars into the primate space upon eruption of lower first permanent molar. Then the first permanent molars are in end-to-end relationship. The closed deciduous arches and straight terminal plane resulted in a transitory end-to-end relationship of the first permanent molar with proper occlusion effected through a late mesial shift of the mandibular permanent first molars.

Baume (p. 338) found lateral and frontal alveolar growth during the time of eruption of the successional incisors in the anterior region. The increase in the intercranial width was greater in the upper arches than in the lower, and greater in arches which had been closed than in those which had been congentially spaced. The greatest impulse of lateral growth was noted in the upper jaw during the eruption of the first incisors and in the lower jaw during the eruption of the second incisors. These studies should be considered, together with

the earlier studies of Baume, on physiological tooth migration.

Levy (p. 349) kept mice on pyridoxine-deficient diets and then studied the jaws. The epithelium of the interdental papillae ulcerated and inflammatory cells filled the ulcerated regions; the alveolar bone showed regression and the mandibular condyle ceased in growth. The mechanism by which these disturbances occur is not discussed, although it is recognized that pyridoxine may function as a coenzyme of several amino acid decarboxylases. The changes were more severe if more protein was added to the diet.

Burstone (p. 358) has reported the effect of x-ray irradiation on the development of the temporomandibular joint of the mouth. In these times of increasing use of irradiation by the x-ray machine, radium, and other radioactive substances, the effects of radiant energy on various tissues and organs is of more than academic injury. It should be remembered that the immediate result is a response to injury, subsequent changes being a sequel of prolonged insidious effects on the cells.

Jacobson and Kesel (p. 364) measured the ammonia nitrogen content of the supernatant fluid and sediment of centrifuged saliva of 40 calculus-present, 40 calculus-free, and 22 periodontal-diseased individuals. Significant higher ammonia nitrogen content of the supernatant fluid from the periodontal-diseased group was observed. Observation of caries and lactobacillus counts in these groups indicated an inverse relationship between caries and periodontal disease. That particular phase is worthy of a sound statistical study on a large group of individuals classified as to type of periodontal disease and evidence of dental caries activity as well as dental caries history.

Jacobson (p. 375), continuing the study of the possible relationship between ammonia nitrogen content of saliva and dental calculus, found negative results. In the present phase of the study no statistically significant difference was found between ammonia nitrogen production of washed salivary sediment from calculus-free and calculus-forming individuals.

Further studies of Jacobson on salivary ammonia and dental calculus (p. 380) indicate that mucin nitrogen decreases while nonmucin nitrogen and ammonia nitrogen increase as saliva stagnates. Saliva from calculus formers contained a greater amount of nonmucin and ammonia nitrogen and formed these types more rapidly during incubation than did saliva from calculus-free individuals. These studies will be used as bases for further investigation of salivary chemistry.

Mitchell (p. 386) produced effects resembling cretinism in hamsters by feeding propylthiouracil. Studies of the periodontium did not reveal any increase in periodontal disease, in the animals. This experimental study tends to confirm clinical observations that periodontal diseases are mainly the results of local environmental factors and that there is no direct relationship between periodontal and thyroid disease.

Lisanti (p. 392) found evidence of hyaluronidase activity in the saliva of 75 per cent of 64 subjects. Twenty of the individuals had periodontal disease and all of these showed hyaluronidase activity. The findings in relation to periodontal disease should be compared with the studies on kallikrein, another "spreading factor" by Sallay and Nador (*J. D. Res.* **29**: 232, 1950). Lisanti also thinks that hyaluronidase may have some role in the "initiation or spread of caries." This problem is worthy of investigation but no evidence is presented that would indicate the hyaluronidase activity in the process of dental caries.

Taylor, Sweeney, and Mahler (p. 396) plainly and frankly discuss the merit and lack of merit of mechanical amalgamators. Three commercially available models are cited. These scientific reports have immediate value to the practicing dentist.

Jansen (p. 401), who reported an apparatus for sectioning calcified dental tissues in 1946, describes another apparatus which permits cutting four sections per millimeter of tooth substances using the center hole in a disc. The need for "serial" sections of undecalcified tissues is great. The possible misinterpretation of structures seen in single sections points up the importance of the serial section technic for three-dimensional interpretations. Observations on isolated ground sections have been subject to question because of the shortcomings of such two-dimensional studies. Jansen's technic, while by no means the final answer, does offer an improvement and an advance toward serial ground sections.

H. B. G. R.

THE ORGANIC ELEMENTS OF THE ENAMEL

IV. The Gross Morphology and the Histological Relationship of the Lamellae to the Organic Framework of the Enamel

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FEW aspects of enamel histology have been the source of so much controversy and speculation as the so-called lamellae. W. D. Miller in 1902 briefly recorded the existence of previously unrecognized organic remains ("Fasern") in enamel following decalcification of ground sections under a microscope. Bodecker (1905) independently discovered the lamella-like appearance of similar organic elements and named them accordingly. Since then, many observers have refused to consider the so-called lamellae anything but cracks, or other artifacts, while some have looked upon these peculiar structures as important metabolic pathways. Others again have classified the lamellae as significant developmental defects which either may be filled by enamel epithelium before tooth eruption (Gottlieb, 1915 and Orban, 1928), by some unspecified organic matter after eruption (Schulz, 1933), or by an entirely hypothetical reparative ingrowth of connective tissue at any time of life (Forshufvud, 1941).

From a morphological standpoint, a great many dental histologists have looked upon the lamellae as elongations or ramifications of the normally existing enamel tufts (Bodecker, 1906, Beust, 1930, Schulz, 1933, Meyer, 1926, Weidenreich, 1926, Gustaphson, 1945, and others).

The origin of the lamellae has been attributed to a poor calcification of the prisms (Miller 1902, Baumgartner, 1911, and Brammer and Brauer, 1926), or prism sheets (Fish, 1933), or to uncalcified remains of interprismatic substance (Meyer, 1926).

Our knowledge of the location and direction of the lamellae is equally contradictory. They have been thought to run as regular bands in the longitudinal direction of the teeth (Faber, 1923), being most prevalent on the approximal surfaces (Gottlieb, 1915), the smooth surfaces in general (Faber, 1925) or, on the contrary, mostly limited to the fissures (Brammer and Brauer, 1926).

The penetration of the lamellae into the dentin claimed by some (Bodecker, 1911, Gottlieb, 1915, Rygge, 1916, Orban, 1928) has been as emphatically denied by others (Meyer, 1926, Gustaphson, 1945).

Their significance has, as already alluded to, been given a varied interpretation, ranging from that of purely abnormal malformations (Brammer and Brauer, 1926, and others) to that of normal, important anatomical channels for metabolism (Beust, 1930) and, finally, to be given the central position in one of the theories on the cause of dental decay (Gottlieb, 1947). It is a regret-

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table fact that most of the above information has been based upon observations of ground sections. This makes it difficult to attach much credence to some of the far-reaching conclusions drawn in the past because, in the routinely prepared and stained ground sections of enamel, a lamella cannot be satisfactorily distinguished from a crack or fracture line which, obviously, may have one of several histories: (1) produced during the development of the enamel, (2) resulted from traumatic injury of mature enamel, especially if the enamel is weakened by caries or cavity preparation, (3) arisen during extraction of the tooth or, (4)—what is practically unavoidable—during the subsequent preparation of the ground section.

Decalcified enamel sections, permitting a reliable distinction between accidental fracture lines and bands of organic matter have, in the past, been rare and inadequate for the study of this problem. The few existing ones, which are familiar from their repeated reproductions, not only fail to show the relationship of the lamellae to the surrounding organic framework of the enamel but have been selected from unusual locations and conditions, favoring the retention of exceptional organic structures despite the use of crude decalcification procedures (Gottlieb, 1947).

During a series of decalcification experiments, designed to isolate the various organic elements normally present in the enamel (Sognnaes, 1948, 1949), the lamellae and similar bands of organic matter have, due to their bulkiness and great frequency, proved to be the least difficult elements to recover in successfully decalcified specimens. From the material studied during the past four years, the following illustrations are believed to represent the most typical and very frequently encountered type of enamel lamellae. Discussion of their gross morphology, distribution, and histological relationship to the surrounding organic framework of the enamel will be based entirely upon the study of decalcified specimens.

MATERIAL AND METHODS

In view of the frequent presence of man-made artifacts in human teeth, it was felt that the study of this problem would have to include observations in some suitable experimental animals. In that way it would be possible to: (1) fix the teeth before their removal from the jaws; (2) select at will animals with unerupted and erupted teeth; and (3) eliminate the accidental fractures caused by cavities, drilling, filling, and extraction of human teeth. Hence, besides human teeth, several hundred rat jaws and the jaws of a total of 13 rhesus monkeys ranging in age from 1 to 4 years,* have been studied. All of the monkeys were caries-free and permitted a comparison of erupted and unerupted teeth. The human as well as the animal specimens were first studied grossly and then either immediately prepared for microscopical sections by methods described elsewhere (Sognnaes, 1947, 1948) or, following decalcification, reexamined grossly under binocular microscope and, after microdissection, prepared for spreads and paraffin sections.

* Tooth development in monkeys this age range corresponds to that of the 5- to 20-year age group in man.

RESULTS

Binocular magnification is advantageous for the study of the three dimensional appearance of the common type of lamella. If one examines anterior teeth of adult people in the mouth or after extraction, it will appear as if the enamel had one or more cracks, running as a rule, in the longitudinal direction of the crown. Upon careful decalcification of such teeth, it is almost invariably observed that acid-resistant organic membranes are left in the space of such cracks (Figs. 1 and 3). Fig. 1 shows what represents a very typical location and size of the type of lamella found in incisor teeth of both man and monkey. Following the acid treatment, the surface cuticle was, in this case, removed by binocular dissection in order to make the underlying lamella clearly visible in depth. In view of the fact that the labial surface of anterior teeth is perhaps one of the most frequent locations for the lamella, especially in the cervical region, it is interesting that this is a rare site of caries as compared to the approximal surface which so often, as in the case shown in Fig. 1, presents large carious cavities.

Fig. 2 indicates that organic bands may be recovered in many locations where enamel prisms have been accidentally exposed by traumatic injury. Around the ill-fitting silicate cement fillings (*F*) where recurrence of caries is known to be comparatively rare, and in the weakened enamel below and between the labial and distal filling, are observed organic bands (*X*) which, except for their location, are morphologically undistinguishable from the more typical, longitudinally oriented lamellae. Mottled teeth, known for their caries resistance, are not spared the lamella, several rows of which are shown in Fig. 3 which shows one of a number of similar mottled teeth obtained from Texas. Fig. 4 shows a great many lamellae in an erupted first permanent molar from a 2-year-old rhesus monkey. On the whole, the study of the animal material indicated that (1) lamellae are as rare in unerupted teeth as they are frequent in erupted teeth, and (2) that the freedom from caries in animals cannot be attributed to any lesser frequency of lamellae in their functioning teeth.

Through binocular dissection, it was possible to segregate and remove the various organic elements of the enamel for microscopical examination as smears and spreads. An intimate attachment was found to exist between the lamellae and surface cuticle of the enamel on the one hand and the underlying prism sheaths on the other. When torn off from the underlying framework and spread on a slide, the enamel cuticle exhibits the histological appearance of crosscut enamel prisms (Fig. 5). If the surface cuticle is thin and delicate, as compared to the lamella, as it usually is, it is difficult to separate one completely from the other. Hence, in Fig. 6 a thin film (*C*) is still attached to the lamella (*L*). The latter varies in thickness but is by and large an homogenous film devoid of structural orientation. As a rule, the external portion of the lamella is thicker than the internal part. This fact, and the great variation in affinity for stain following decalcification, gives rise to the uneven density of the spread of the lamella seen in Fig. 7.

Fig. 1. Fig. 2.

Fig. 3. Fig. 4.

Figs. 1 to 4.—Gross appearance of organic membranes in enamel of erupted teeth. Following demineralization, the surface cuticles and external organic framework were removed by binocular dissection. Specimens were immersed in water to prevent collapse of organic bands during photographic exposure. Reflected light. (×6.)

Fig. 1.—Mandibular incisor with distal cavity and grossly visible labial lamella (*L*) extending complete length from incisal edge to enamel-cementum junction, and from surface to dentino-enamel junction.

Fig. 2.—Maxillary canine, partly demineralized. Heavy organic bands, or cavity membranes (*C.M.*), are seen surrounding the ill-fitting distal and labial silicate fillings (*F*). Organic bands (*X*) are also noted in the enamel between and cervical to the fillings, presumably resulting from cracks produced during or following cavity preparation.

Fig. 3.—Incisor affected with moderately severe mottled enamel (from material obtained in Texas). Numerous lamellae but no caries can be observed.

Fig. 4.—Maxillary first permanent molar of caries-free, 2-year-old rhesus monkey. Numerous cracks, which could be seen by binocular examination of this and many similar specimens before decalcification, revealed themselves as bands of organic matter (lamellae) once specimens had been decalcified.

In order to study the histological relationship of the lamella to the surrounding organic framework of the enamel, it is necessary to prepare thin paraffin sections of carefully demineralized specimens. Some of the facts which thereby could be established are exemplified by the composite illustration shown in Figs. 8 and 9.

In the first place, it became evident that the lamellae (*L*) do not represent uncalcified prisms as has been a widely accepted concept in the past. Where the lamella has been cut in a nearly perfect cross section (Fig. 9), its true location is seen to be between the prisms, corresponding fairly well to the combined prism sheaths and interprismatic substance. Second, it is of considerable interest that the lamella neither runs a straight course nor follows any one prism, or group of prisms (Fig. 8), which again indicates the independence of the lamella from the prism development. It has already been mentioned that the lamellae are generally more bulky in the external portion of the enamel. More often than not a lamella will not reach the dentin and if it ever enters the dentin such a case must be too unusual to occur in the average type of freshly prepared teeth.

The absence of organic lamella-like elements in unerupted teeth would seem to suggest that the lamellae are not normal or usual developmental characteristics of enamel.

Observations of several hundred human teeth have, after successful decalcification, shown that the lamellae and similar organic membranes are apt to be found where functional stresses of one kind or another have caused discontinuity between the enamel prisms.

It is perhaps not surprising that functioning teeth often suffer from mechanical trauma of the enamel and, after careful decalcification, it has been found that the internal enamel surfaces exposed by such injury are covered by organic membranes. There is rarely found a "naked" enamel surface exposing the mineral component of the enamel prisms to the oral cavity. Heavy organic bands can be found surrounding cavities, penetrating spaces left by cracks and fissures, and lining the enamel walls of cavities, filled as well as untreated ones. Successfully decalcified specimens have shown that the cavity walls in the enamel of carious teeth usually are heavily fortified with a pigmented membrane firmly attached to the delicate layer of the underlying white normal enamel matrix. The pattern of these cavity wall membranes is determined by the degree of corrugation of the enamel, that is, the course of the longitudinal rows of prism bundles (Sognnaes, 1948). Where Schreger's bands are marked, the organic bands are found to follow sharply the alternating bands in the fashion of a corrugated ribbon.

These various organic membranes share certain common characteristics. They do not follow single prisms nor groups of contemporarily formed prisms. Neither is there any consistent relationship between the incremental formation of the enamel and the course of the organic membranes described above.

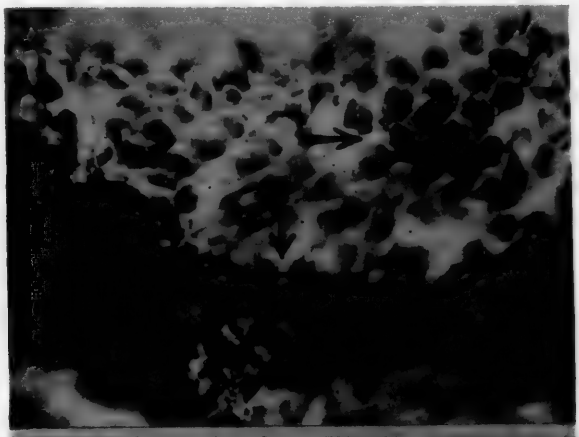

Fig. 5.

Fig. 6.

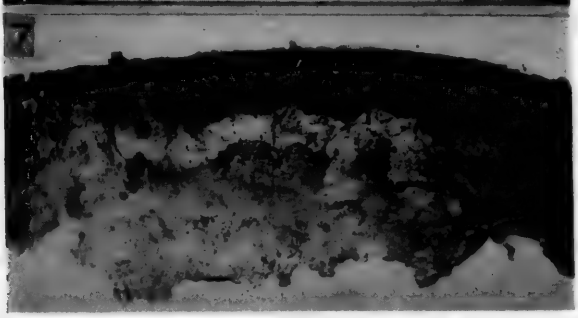

Fig. 7.

Figs. 5 to 7.—Surface cuticle and lamellae removed by binocular dissection and prepared as spreads for microscopical examination in transmitted light.

Fig. 5.—Enamel surface cuticle shows imprint of enamel prisms where torn off from underlying organic framework of the enamel. H. & E. stain. (×1000.)

Fig. 6.—Lamella (*L*) with parts of surface cuticle (*C*) attached. Uneven thickness causes light and dark areas: otherwise, lamella appears as a structureless, homogenous membrane. Unstained. (×20.)

Fig. 7.—Heavy lamella showing increasing thickness from internal portion to curved external surface. Great variation in affinity for stain results in part from decalcification process and could not be correlated with any specific structural pattern.

DISCUSSION

The developmental origin of the lamellae, suggested by Gottlieb and his pupils, is not supported by this study because of: (1) the failure of the lamella to follow the pattern of developmental structures and developmental pathology of the enamel, and (2) by the failure to find lamella in unerupted teeth with a frequency which comes anywhere near that in erupted teeth. Illustrations which are claimed to show epithelial cells and hornified epithelium inside the enamel of developing teeth must be classed as unusual. No hornification has been observed in the external portion of lamellae when successfully decalcified and sectioned in proper relationship to the surrounding enamel framework. The external hornification suggested by an illustration by Orban might well be due to a fold of the surface cuticle, as indicated by the collapse of the lamella following unsuccessful recovery of the supporting prism sheaths. This is not the only technical problem which may influence the microscopical appearance of a lamella, or crack, as the case may be. Since a crack has a tendency to occur along lines of least resistance, that is, along the prism sheaths, it is quite understandable that accidental cracks will provide more direct access from the outside to the organic constituents of the enamel facing the crack opening. Such regions are, in general, more accessible than intact enamel to stains, as well as to fixatives and decalcification fluids, used in preparation of sections.

As pointed out before (Sognnaes, 1948), it is believed that the difficulty of staining, fixation, and decalcification of the enamel in general is due to the fact that the organic matter normally present in the fully mature and intact enamel of erupted teeth is so infiltrated and petrified by calcified matter that it is virtually inaccessible to histological reagents. In the neighborhood of a crack, on the other hand, the chemicals will come in much closer contact with a wide area of organic prism sheaths which, even within the thickness of a longitudinal ground section, would embrace half a dozen prism surfaces.

It is well known that organic surface cuticles of the enamel are comparatively easy to recover after decalcification. Such surface membranes obviously offer the best access to fixatives and acids. This is probably also the explanation of the organic films which Miller (1902), after successive grindings, repeatedly recovered from the very surface layer of ground sections of enamel.

On the basis of these considerations, it is suggested that a simple crack in the enamel, in general, is more favorably exposed to fixatives as compared to the rest of the enamel and, in addition, is easier to stain and decalcify. Even if the rest of the organic matter of the enamel were to be lost one would, therefore, expect to recover a band of organic matter in the region of uncomplicated cracks or fracture lines. In line with this reasoning, it may, furthermore, be suggested that a simple crack in the enamel, even if occurring after removal of the tooth from the jaw, might present itself in a section as a delicate organic band indistinguishable from a thin lamella. The frequent presence of organic bands thicker than the width of the prism sheaths could be explained on a similar basis if it were assumed that organic matter may diffuse into cracks, either from the outside or from the inside. Since it has been found that lamellae

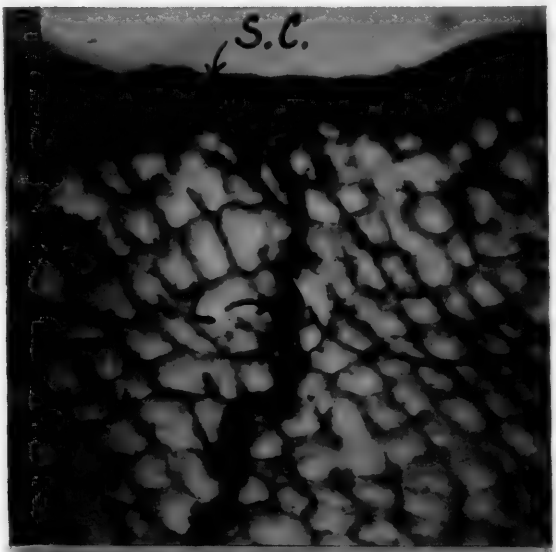

Fig. 8.

Fig. 9.

Figs. 8 and 9.—Paraffin section of successfully decalcified enamel of human molar showing the relationship between lamella and surrounding organic framework of enamel. From surface cuticle the lamella is seen to proceed along an irregular course between enamel prisms. Where cut tangentially, it is noted that the lamella is related to regions between the prisms, not to uncalcified prisms. H. & E. stain. (×1000.)

in unerupted teeth are so extremely rare as compared to lamellae in erupted teeth, saliva would seem to be one source of organic matter which most readily could reach and fill any cracks of the enamel wide enough and with sufficient external opening to permit penetration of protein compounds.

It has been a common impression that the enamel lamellae are detrimental developmental defects. This study does not indicate that the common lamellae are developmental defects and, until more evidence is forthcoming, one should perhaps not overlook the possibility that they may represent a crude form of repair of enamel following traumatic injury of this unique tissue which is so devoid of the usual mechanism of repair. It has been mentioned that in any exposed enamel surface, whether it be exposed by grinding, by cavity preparation, by caries, or by cracks, it is, as a rule, found that an organic lining can be recovered from these areas after successful demineralization. Since formation of calcified matter is common around the teeth, in the form of calculus, it is perhaps also feasible that minerals may infiltrate the organic matter in a crack. Hence, the lamellae rather than being detrimental might serve as a nucleus for mineralization and thus provide a much needed repair of the enamel cracks which, probably, are the most common injuries affecting the enamel of otherwise healthy teeth of animals and man.

SUMMARY

1. The enamel lamellae have been studied grossly, in spreads, and in paraffin sections of successfully demineralized teeth from rats, monkeys, and man.

2. In erupted teeth organic bands are very frequently found in regions where the enamel has suffered from traumatic injury. The failure of the lamellae to follow the pattern of enamel formation and their absence in unerupted teeth contradict the belief that the lamellae are formative defects.

3. The lamellae are found in the region between the enamel prisms corresponding to the prism sheaths (areas of least resistance) and do not consist of uncalcified prisms.

4. It is suggested that the organic membranes which infiltrate and cover prism surfaces exposed by traumatic injuries to the enamel may serve as a nucleus for a crude reparative mineralization of such defects.

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THE INFLUENCE OF SOME SURFACE-ACTIVE SUBSTANCES ON DECALCIFICATION OF THE ENAMEL SURFACE

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THE POSSIBILITY of using salts or other substances to protect tooth enamel against decalcification has long been recognized. In recent years many compounds have been studied for their power of reducing enamel solubility, and this trend in dental research is well exemplified by the work of Muhler, Boyd and Van Huysen,[1] who examined 145 substances, and of Manly and Bibby,[2] who reported results for 147. This method of protection appears to operate by reaction of the applied material with the calcium phosphate of the tooth surface, either by adsorption or by ion exchange, to convert it into a new substance of lower solubility. The data of the latter authors suggest that the most effective reagents may be fluorides or salts of some of the rarer elements.

A less radical way of protecting the tooth surface is suggested by several reports of reduction of caries resulting from diets with a high fat content. Rosebury and Karshan[3] in 1939 found that the addition of various fats and oils to the diet of the cotton rat gave decreasing levels of fissure caries. Schweigert, Shaw, Zepplin, and Elvehjem,[4] also working with the cotton rat, found that diets to which lard was added at the expense of sucrose gave much greater protection than could be accounted for merely by the sucrose reduction. These authors said that "The effect of the fat may be 'physical' in that it forms a protective coating over . . . the teeth." Similar views have been expressed by Box,[5] and by McCollum, Orent-Keiles, and Day,[6] who consider that fat may waterproof the tooth surface so that acid is prevented from coming in contact with the enamel. Box also showed that both whole teeth and powdered enamel could be markedly protected by oleic acid against decalcification in vitro.

This paper describes an investigation made to determine to what extent an intact tooth surface, already wet with saliva, could be covered with a protective film. The substances examined were oily or surface-active materials which had no strong chemical action on the enamel.

EXPERIMENTAL METHODS

The stability of any oily or greasy protective film will depend upon its power of adhering to the given surface in competition with the decalcifying medium. This poses complex problems involving surface energies and viscosities and makes it desirable that experiments in vitro should depart as little as possible from conditions obtaining in the mouth. For this reason, acidified saliva was preferred to simple aqueous buffer solutions and was allowed to act on whole teeth at 37° C.

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Pooled human saliva was obtained by interposing a trap in the saliva ejector in use on patients undergoing conservative treatment. The product was allowed to stand overnight in the refrigerator and decanted from any solids which had separated to the bottom. A suitable lactic acid-sodium lactate buffer, 2.5M with respect to total lactate radical, was then added to adjust the saliva to the required pH and to make it 0.2M in total lactate. Sound human teeth were kept in 10 per cent formalin solution until required.

TABLE I

WEIGHT OF CALCIUM DISSOLVED FROM WAXED TEETH
(10 ml. saliva at pH 4.0 for 20 hours)

First side (mg.)	2.5	3.1	3.3	3.0	2.8	2.9	2.3	2.3	3.8	2.6
Second side (mg.)	3.4	3.4	3.6	3.8	2.8	3.4	3.0	3.3	3.9	3.1
Difference (d)	-0.9	−0.3	−0.3	−0.8	0.0	−0.5	−0.7	−1.0	−0.1	−0.5

Mean difference $\bar{d} = -0.51$ mg.
Standard error of $\bar{d} = 0.107$ mg.
Probability of occurrence by chance < 1 per cent

To evaluate the protective action of any agent it becomes necessary to compare the extent of decalcification on two tooth surfaces, one pretreated with the agent and one used as a control. As it is well known that teeth differ widely in their solubility in acid, it was decided to carry out the control experiment on the same tooth. A preliminary experiment was made as follows:

Teeth were rinsed in acetone and allowed to dry in the air. One-half of the enamel surface and the whole of the root of each were then coated with sticky wax, the exposed surface was wetted with normal saliva, and each tooth was immersed in 10 ml. buffered saliva at pH 4.0 in a stoppered tube. The tubes were placed in an incubator at 37° C. for 20 hours with frequent agitation. At the end of that time the teeth were removed, rinsed successively in water and acetone, and stripped of wax with four changes of boiling carbon tetrachloride. They were then waxed again, so that the second side was exposed, and were treated with fresh acid saliva as before. The increase in calcium content of the saliva, measured by the method of Clark and Collip,[7] represents the calcium dissolved from the unwaxed area of the tooth. If this procedure of dividing the enamel surface by waxing is to be of any value, the weights of calcium dissolved from the two sides should be virtually the same. Table I shows the first results obtained from a group of ten teeth.

TABLE II

WEIGHT OF CALCIUM DISSOLVED AFTER PRELIMINARY WAXING
(10 ml. saliva at pH 4.0 for 20 hours)

First side (mg.)	4.4	2.7	3.3	3.2	3.1	3.1	2.7	2.7	3.0	3.0
Second side (mg.)	4.2	2.8	3.7	2.7	2.5	3.1	2.4	3.1	3.7	2.7
Difference (d)	0.2	−0.1	−0.4	0.5	0.6	0.0	0.3	−0.4	−0.7	0.3

Mean difference $\bar{d} = 0.03$ mg.
Standard error of $\bar{d} = 0.135$ mg.
Deviation from zero is not significant.

Fisher's "t" test has been used to show that the probability of the observed mean difference occurring by chance is less than 1 per cent. This implies that waxing and stripping the tooth in some way modified the second exposed side,

making it more susceptible to attack by acid. In an attempt to overcome this difficulty, another ten teeth were given a preliminary treatment by coating completely with wax and stripping with carbon tetrachloride. They were then waxed and decalcified on alternate sides as already described. Table II shows that this time there was no significant difference between the two sides.

Method I.—On the basis of these experiments, a method was devised for testing the effect of added substances on decalcification. Teeth were dried with acetone, completely waxed, and stripped with carbon tetrachloride. Each was again coated with wax, only one-half of the enamel being left exposed. The teeth were thoroughly wetted by immersion in normal mixed saliva for ten minutes, the substance under test was applied by brushing, and each tooth was placed in a numbered stoppered tube holding 10 ml. saliva at pH 4.0. These tubes were kept at 37° C. for 20 hours with frequent shaking, after which the teeth were removed, rinsed, stripped with carbon tetrachloride, and waxed again leaving the second half of the enamel surface exposed. After wetting with normal saliva, the teeth were subjected to decalcification in fresh acid saliva as before, but with the test substance omitted. The degree of protection afforded by the test substance was calculated from the expression,

$$\text{Protection (per cent)} = \frac{\text{mg. Ca dissolved in control} - \text{mg. Ca dissolved in test}}{\text{mg. Ca dissolved in control}} \times 100$$

Ten teeth were used in each experiment and it was found that, provided the preliminary waxing was carried out, the results were not affected by the order in which the control and test measurements were made.

The pH of the saliva was measured with a glass electrode at the end of the decalcification period and was found in every instance to have increased by about 0.20 irrespective of the nature of the protective agent.

Method II.—The usefulness of the above method is dependent upon the precision with which the enamel surface can be divided into two equal areas by waxing. Hence, it is best applied to anterior teeth where the more regular surfaces are more easily compared. To study similar effects for all kinds of teeth a slightly different method was adopted. The roots only of teeth were waxed, so that the whole enamel surface was exposed. The teeth were then sorted into several groups, each of thirty teeth, so that the members of each group matched those of the other groups as closely as possible in type and size. Each group was used to test a different protective agent, one group being employed as a control. Each tooth was treated with 10 ml. of the same acid saliva for 20 hours, the test substance having been applied as before, and the increase in calcium content of the saliva was determined. The results were then submitted to a statistical examination and the extent of protection was evaluated by comparison with the control group.

RESULTS

Preliminary qualitative experiments showed that fats, such as butter or lard, and greasy substances, such as petroleum jelly, afforded the tooth some small measure of protection, whereas their liquid counterparts, such as olive oil

and paraffin oil, appeared to be almost ineffective. The action of the more viscous group was irregular and probably derived from mechanical properties rather than from any affinity for the tooth surface. The inquiry was therefore extended to less common compounds which might be expected to form an adsorbed film.

The observation of Box concerning the behavior of oleic acid was confirmed, but much better results were found with long paraffin chain amines. Of these, cetylamine* or hexadecylamine, $C_{16}H_{33}NH_2$, will be considered here, although other members of the homologous series were found to give similar protection. Cetylamine is a waxlike solid melting at 46° C., so that in this work it was used in the form of a 1 per cent solution in medicinal paraffin oil. The solubility of cetylamine in paraffin is evidently low, as the solution becomes cloudy at room temperature, although it remains clear if kept at 37° C. Fig. 1 shows several teeth treated on one side with various oils. The only one visibly protected is that which received cetylamine in paraffin, although oleic acid has a definite but smaller effect.

Fig. 1.—Teeth etched on right side for six hours in saliva at pH 4.0. Left side treated similarly in the presence of (*A*) no protective agent, (*B*) oleic acid, (*C*) paraffin oil, and (*D*) cetylamine in paraffin oil. Note good protection with cetylamine.

Using ten teeth for each substance, several oils and solutions were tested by the first quantitative procedure described above, with the results shown in Table III.

TABLE III

PROTECTIVE EFFECT OF VARIOUS AGENTS AGAINST SALIVA AT pH 4.0 FOR TWENTY HOURS
(Averages for ten teeth)

AGENT	PROTECTION (PER CENT)*
Paraffin oil	Not significant
Olive oil	Not significant
Oleic acid	17.8 ± 8.4
Cetylamine in paraffin	49.0 ± 9.4
Cetyl alcohol in paraffin	Not significant
0.1 per cent aqueous sodium lauryl sulphate	Not significant

*Showing 95 per cent confidence interval calculated from Fisher's table of "t" for 9 degrees of freedom.

*The cetylamine used was manufactured by Armour and Company, Chicago, under the name of Armeen 16D.

Since cetylamine in paraffin was by far the best agent examined, more complete tests were made to measure its dependence on experimental conditions. To obtain some information concerning the permanence of the protective film, an experiment was made beginning with 15 ml. acid saliva per tooth and taking samples at intervals. Table IV shows the results for ten teeth, the protection for each period being calculated from the weights of calcium dissolved during that period and not from the beginning of the experiment.

TABLE IV

PROTECTIVE EFFECT OF CETYLAMINE IN PARAFFIN OIL
(Averages for ten teeth)

TIME (HOURS)	PROTECTION (PER CENT)*
0-2	73.2 ± 6.5
2-4	58.6 ± 11.6
4-8	50.5 ± 11.5
8-24	30.6 ± 11.7
Mean 0-24	47.0 ± 9.2

*Showing 95 per cent confidence interval calculated from Fisher's table of "t" for 9 degrees of freedom.

The second quantitative method was used in a study of the action of cetylamine at two different hydrogen-ion concentrations and in oils of two different viscosities. The agents used were: (a) Medicinal paraffin oil of viscosity 440 sec. Redwood at 37° C. (b) one per cent cetylamine in light paraffin oil. Viscosity of the solution approx. 100 sec. Redwood at 37° C. (c) one per cent cetylamine in (a). Viscosity of solution 520 sec. They were tested against saliva at pH 3.2 and pH 4.0, and a control experiment was also made at each pH level. This required eight lots, each of thirty teeth, and 10 ml. saliva per tooth. It was impossible to run so large an experiment at one time, and so each lot of thirty teeth was divided into six groups of five. The experiment was then run in six sections, each extending over the whole eight treatments and each using a new batch of saliva. The experimental results are shown in Table V. It is evident on inspection that different batches of saliva produced widely different degrees of decalcification. For this reason the data at each pH level were submitted to analysis of variance to obtain an estimate of the standard error of the treatment means.

Table VI disposes of the large component of variance arising from differences between batches of saliva and provides the best estimate of error for establishing the confidence intervals of Table VII. As would be expected, it also demonstrates that the group-treatment interaction is of scant significance.

DISCUSSION

Of the substances studied here, only two have been found to form a stable protective film under the conditions of the experiment. Such different oils as paraffin and olive oil applied to a wet tooth have no significant influence on the rate of decalcification. Addition of only 1 per cent of cetylamine to paraffin, however, appears to give it a strong affinity for the tooth surface, so that at pH 4.0 one application can reduce decalcification by as much as 50 per cent over a 20-hour period.

TABLE V

WEIGHT (MG.) OF CALCIUM DISSOLVED IN TWENTY HOURS

	pH 3.2				pH 4.0			
			CETYLAMINE				CETYLAMINE	
GROUP	CONTROL	PARAFFIN	IN LIGHT PARAFFIN	IN MEDICINAL PARAFFIN	CONTROL	PARAFFIN	IN LIGHT PARAFFIN	IN MEDICINAL PARAFFIN
	8.4	9.8	6.8	5.3	5.0	6.1	4.3	2.5
	10.7	8.2	7.1	5.1	4.4	5.1	2.0	2.5
1	7.4	12.8	12.5	8.6	4.9	6.7	3.6	1.8
	12.6	12.9	12.4	8.0	6.8	5.2	2.5	2.3
	12.5	10.6	4.6	5.2	3.9	4.2	2.4	2.0
	10.8	12.7	8.5	9.9	3.2	4.0	2.8	2.0
	11.7	13.2	9.1	10.0	3.1	4.0	2.8	2.3
2	14.6	12.0	14.0	8.6	4.0	4.3	4.0	3.2
	10.2	11.0	9.5	10.6	4.2	4.4	3.2	2.3
	14.0	16.0	12.3	7.4	3.2	3.7	2.7	2.4
	5.7	8.8	6.1	7.4	5.7	5.4	2.6	1.4
	8.1	10.9	5.7	7.2	3.7	5.1	3.4	2.4
3	8.1	8.1	4.8	6.7	3.1	3.8	2.6	1.5
	9.6	8.8	8.1	6.4	4.4	3.2	2.3	1.6
	9.3	11.0	7.6	7.5	1.4	4.3	2.5	2.0
	12.8	11.2	11.7	11.5	3.3	3.4	2.2	1.7
	11.2	10.1	9.4	7.4	4.6	4.7	3.4	2.3
4	9.8	11.8	11.0	7.3	4.6	5.6	3.5	1.6
	10.6	9.1	8.1	7.2	3.9	3.3	2.8	1.9
	12.1	11.4	9.5	7.6	3.9	4.9	2.7	1.9
	11.8	10.4	13.8	8.2	5.5	4.2	2.7	2.1
	13.4	15.8	8.1	5.7	4.0	4.4	3.5	2.0
5	14.4	13.0	8.9	11.7	5.0	3.4	3.3	1.5
	12.4	12.1	9.5	4.6	3.7	5.4	2.5	2.3
	12.4	12.8	11.3	8.8	3.8	4.7	2.6	1.4
	12.7	19.6	10.4	11.4	5.6	6.1	2.6	1.7
	14.7	13.5	9.7	10.1	6.1	6.8	2.7	1.7
6	18.3	16.5	10.4	11.2	6.1	6.1	3.4	2.6
	15.9	13.8	9.5	7.2	6.0	6.3	4.5	2.1
	16.2	11.6	11.9	9.7	6.8	5.7	3.3	1.9
Mean	11.75	11.98	9.41	8.12	4.46	4.82	2.98	2.03

TABLE VI

ANALYSIS OF VARIANCE OF DATA OF TABLE V

COMPONENT OF VARIANCE	SUM OF SQUARES	DEGREES OF FREEDOM	MEAN SQUARE	F.
	pH 3.2			
Total	1035.91	119		
Among groups	302.46	5	60.49	16.17
Among treatments	315.53	3	105.18	28.12
Group-treatment interaction	59.43	15	3.96	1.06
Within group treatment (error)	358.49	96	3.74	
	pH 4.0			
Total	243.22	119		
Among groups	23.80	5	4.76	9.56
Among treatments	152.19	3	50.73	101.8
Group-treatment interaction	19.45	15	1.30	2.61
Within group treatment (error)	47.78	96	0.498	

TABLE VII
DEGREE OF PROTECTION BY CETYLAMINE IN PARAFFIN

AGENT	PER CENT PROTECTION*	
	pH 3.2	pH 4.0
Medicinal paraffin only	Not significant	
Cetylamine in light paraffin	19.9 ± 8.7	33.2 ± 8.3
Cetylamine in medicinal paraffin	30.8 ± 8.7	54.5 ± 8.3

*Showing confidence interval at 95 per cent level.

The data of Table VII show that greater protection was given by the more viscous solution of cetylamine and that both were less effective at the lower pH. Replacement of the $-NH_2$ group of cetylamine by an -OH radical, as in cetyl alcohol, completely destroys the protective effect. Nevertheless, although the $-NH_2$ group is basic it is clear that the effect of the amine is not merely one of neutralizing acid, since the amount applied to an average tooth (about 1 to 2 mg.) is negligible compared with the quantity of lactic acid present in the buffered saliva and, indeed, the final pH was independent of the agent used. Moreover, the only other substance found to give any protection was oleic acid.

A tentative explanation of the behavior of paraffin solutions of cetylamine may be put forward. It seems probable that the $-NH_2$ groups of the amine are adsorbed at the enamel surface and the hydrocarbon radicals of the cetylamine then act as a bridge between the enamel and the paraffin oil, which is enabled to form a more or less stable film. The affinity between enamel and amine is sufficient to permit the displacement of saliva from the tooth surface, a process which is facilitated by brushing, and once established the more viscous oils are less easily removed. Work is proceeding with the object of studying nore fully the influence of various polar groups, of chain length, and of physical properties of the oil.

Since it is generally agreed that decalcification of tooth surfaces by acid is the initial lesion in dental caries, the application of oily adsorbed films suggests itself as a possible prophylactic measure. The conditions of test reported here were in two respects more severe than normally occur in the mouth; the pH was lower, and this acidity of the saliva was maintained for 20 hours. For shorter periods decalcification was inhibited by as much as 70 to 80 per cent. On the other hand, although the test samples were agitated at frequent intervals, the mechanical influences which may tend to destroy the film were possibly much weaker than would be met with in the oral cavity. However, it is of significance that the greatest incidence of caries is in areas of stasis where mechanical disturbance to an adsorbed protective film would be minimal. The clinical value of long chain aliphatic amines in preventing dental caries is subject to further experiment, but the results in vitro are encouraging.

SUMMARY

1. A number of surface-active and oily materials have been investigated to determine their ability to protect wet tooth surfaces against decalcification by acid saliva.

2. Two experimental methods have been developed and shown to give comparable results.

3. Of the natural and mineral oils studied, measurable protection was observed for only two, namely, oleic acid and solutions of long chain aliphatic amines in paraffin oil.

4. Over a 2-hour period, a single application of cetylamine in paraffin to the wet tooth afforded 70 to 80 per cent protection against saliva at pH 4.0. When the action of the acid was prolonged to 24 hours, the protection was still of the order of 50 per cent.

5. Some of the conditions governing the action of cetylamine solutions have been examined.

The authors wish to express their indebtedness to Mr. J. Rogers of the Otago School of Mines for making available a wide range of surface-active agents, and to the many members of the profession who supplied the sound teeth used in this investigation.

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THE WEIGHT LOSSES OF INTACT TEETH IN DIFFERENT BUFFERS AT VARYING pH VALUES

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IN THE appraisal of decalcification phenomena against the background of the caries problem, both the new and the old theories agree that acids are detrimental to tooth structure.

According to the traditional conception, the acid nature of caries is indisputable. The opponents of this theory, although maintaining that caries initiation is determined solely by the passability of the organic channels, pay full recognition to the injurious effect of carbohydrate fermentation and readily admit of the acid component in the carious process.

Though slow acid action may appear to be beneficial, in that it erects a barrier against the spread of caries invaders, a fairly high concentration of H ions may subsequently overcome the resistance of this barrier. Thus, a quantitative study of acid solvent action on tooth structure was thought justified; the study of the conditions of acid attack is especially indicated, as some of them are conditions actually found in the mouth, in our consuming of fruit juices and commercial soft drinks, or in the mouths of pregnant women, with 81 per cent of their salivary glands secreting saliva that is acid in reaction at the mouth of the ducts.[1]

MATERIALS AND METHODS

Intact teeth, freshly extracted or kept in stock, were cleaned and washed under running water, and then dried with blotting paper; before placing them in the various buffer solutions, they were weighed on an analytical balance. The method employed was that of Phillips and Muhler.[2]

In addition to whole teeth, powdered enamel and dentin were also used in our experiment for comparison. Powdered cementum was not used as the cementum of the root is exposed in the oral cavity only at an advanced age with the recession of the gingivae. The powdered enamel and dentin were prepared according to Manly and Hodge[5] to pass a 200-mesh screen.

The loss of weight of powdered enamel and dentin was determined in filtering crucibles, according to the standard technics corresponding to that of Buonocore and Bibby.[3]

The buffers used were mainly lactate and citrate. A few teeth were also kept in the phthalate and tartrate buffers. Buffers of 2.3 to 5.0 pH were prepared as follows, according to Michaelis[4]: Lactate buffer: lactic acid, 0.1 N to 500 c.c. Sodium lactate, 0.1 N to 500 c.c. Citrate buffer: 21.008 Gm. crystalline citric acid + 200 c.c. NaOH 0.1 N.

The amount of buffer used in each experiment was 40 c.c. of solution to one gram of tooth weight. The pH values were checked with the aid of a Beckman

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pH meter and glass electrode. The buffer solutions were renewed at regular short intervals. The rise in pH was due to minerals drawn from the tooth structure.

The initial loss of structure and the influx of its minerals into the solution is probably not recorded through change of pH, since this change is compensated by the buffer capacity of the reagent. Only when the "perfusion" of minerals was in excess of the buffer capacity could a raise in pH be noted.

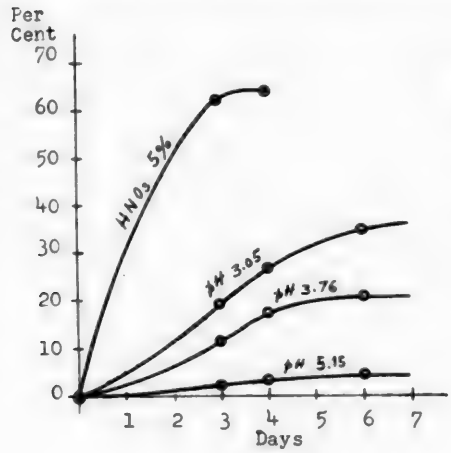

Fig. 1.

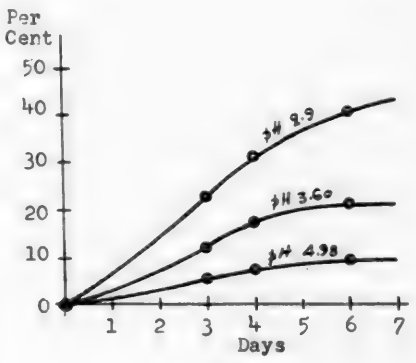

Fig. 2.

Fig. 1.—Lactate Buffer Series I at 37° C. Percentage of loss of weight in relation to time.
Fig. 2.—Citrate buffer Series I at 37° C. Percentage of loss of weight in relation to time.

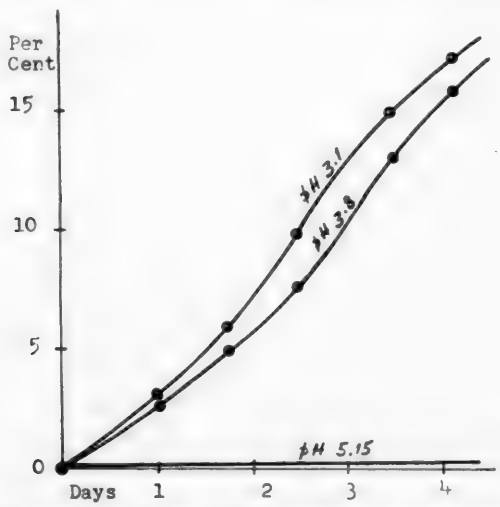

Fig. 3.—Lactate buffer Series II at 20° C. Percentage of loss of weight in relation to time.

The experiment was carried out in a thermostat in two series, one at 37° C., the second at 20° C.

Results are given in Figs. 1-9.

DATA

Figs. 1 to 4 represent data of loss of weight with relation to time, at 20° and 37°, and at a relatively constant pH. Figs. 6 to 8 provide correlation of loss of weight versus pH, with time constant. Fig. 9 deals with individual acid buffers in terms of change of pH versus time.

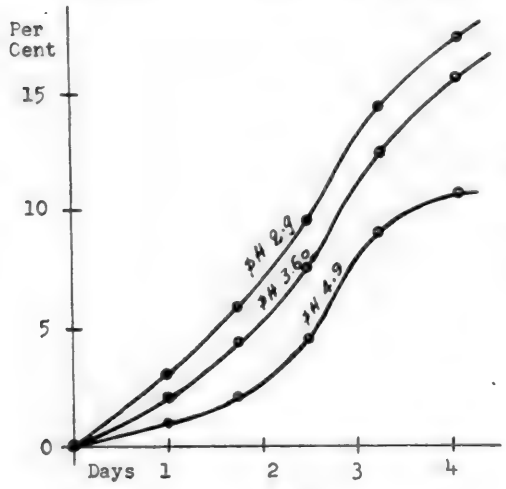

Fig. 4.

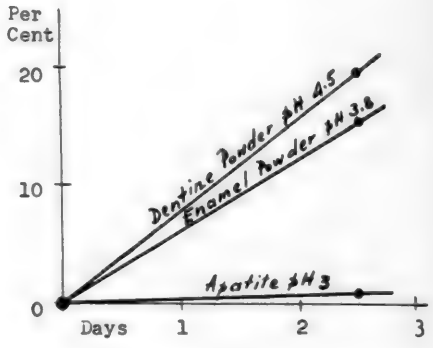

Fig. 5.

Fig. 4.—Citrate buffer Series II at 20° C. Percentage of loss of weight in relation to time.
Fig. 5.—Compared loss of weight of dentin, enamel, and apatite at 20° C.
The attack is linear as the matter is homogeneous.
Original pH 2.4 in all solutions.

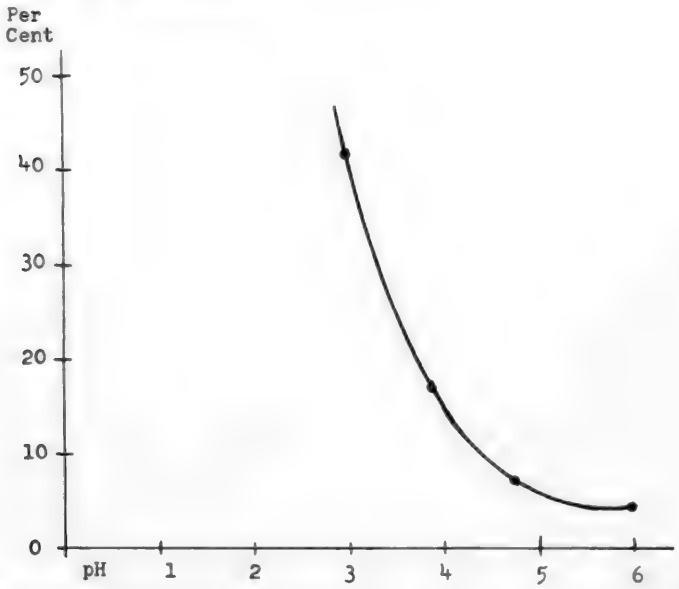

Fig. 6.—Lactate buffer at 37° C. Percentage of loss of weight versus pH.

The experiment with 5 per cent nitric acid (Fig. 1), routinely used for decalcification in microscopic technics, was designed particularly to gain an insight of loss of weight with time in the form of a standard curve. It was planned to repeat this experiment checking the change in pH values at half-hour intervals and correlating these changes with loss of weight of tooth structure. Conditions of war in Israel thwarted further work.

DISCUSSION

The analysis of the curves permits the conclusion that the rate of dissolution of tooth structures is in main a linear function of hydrogen-ion concentration. In Figs. 6, 7, and 8, the pH was plotted on the abscissae. If the actual normality of $[H^+]$ is used instead, reasonably straight lines are obtained. Rise in temperature is an additional factor affecting dissolution.

The loss of weight does not appear to increase linearly with the time of exposure, i.e., the decomposition does not proceed at a constant rate, but shows rather an S-shaped curve characteristic for each buffer.

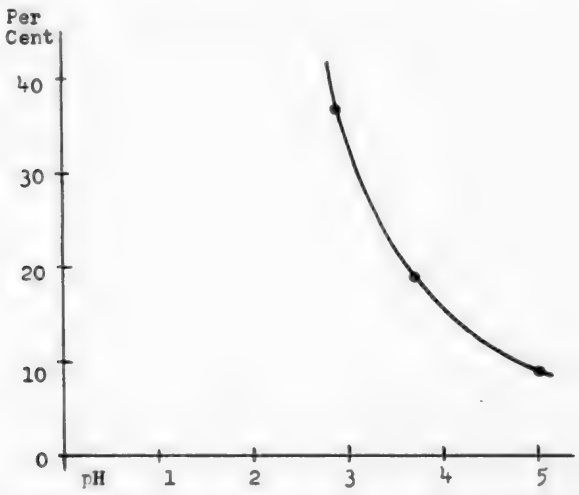

Fig. 7.—Citrate buffer at 37° C. Percentage of loss of weight versus pH.

With all buffers, the attack is at first slow, the enamel alone being dissolved. Dissolution then proceeds with greater rapidity after the diffusion of acid through the denatured protein of the organic channels has reached the dentin and the combined surface areas, both of enamel and dentin, become exposed to an acid attack. Then the curve regularly flattens. At pH 5.15 there is practically no attack. (Figs. 3 and 7.)

The initial lag period in the dissolution of tooth substance deserves special attention, as it seems to represent a threshold necessary to overcome some kind of a biological barrier against acid diffusion, probably due to the continuous organic matrix and its even distribution within the enamel.[7] Clinically, it may be

of great importance because, under conditions prevailing in the mouth, it may allow time for the saliva buffers to compensate a sudden drop of pH and thus prevent immediate damage to the teeth.

If the weight losses are plotted against pH (Figs. 6 and 8), it may be observed that when approaching neutrality, the buffer attack sinks asymptotically to zero; the slight dissolution may involve the carbonate fraction of enamel. On the other hand, with decreasing pH, the dissolution steadily increases, up to a certain critical point; the further loss is very slight, either because of decrease of rate of attack with the loss of minerals by the tooth structure, or because of change of electrical charge at the isoelectric point of the tooth at a certain low pH value.[8, 10]

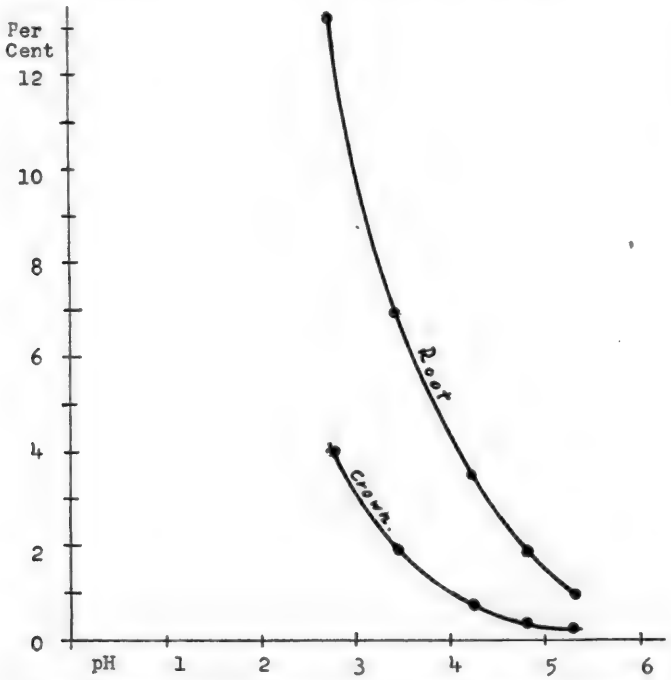

Fig. 8.—Lactate buffer. Percentage of loss in weight versus pH.

Fig. 5 exhibits particular properties strikingly different from the curves of dissolution of whole teeth. It concerns powdered enamel, dentin, and apatite, with a linear course of the process of dissolution. It may be due to the fact that the material dealt with here is more homogeneous. In this case the constants of the straight line may be used not only as a measure of the H-ion concentration, but also as a criterion for the various interaction ability of a definite acid with a typical structure. The enamel is apparently less soluble than the dentin (the apatite is attacked least of all) as it is more compact.

In the experiment with the crown and root separated (Fig. 8) it may be noted that the loss of weight of the crown was considerably less than of the root

of the same tooth, a fact which is explained on the basis of the denser structure of the enamel and its lesser solubility. In the same order and in complete accordance with literature[6] is our finding that powdered enamel samples showed smaller losses than powdered dentin.

The citrate buffers (Figs. 2 and 4) at higher pH values show a stronger attack as compared with the lactate buffer. This abnormal reaction involving citrate may be due to the formation of a little ionizable soluble calcium citrate complex which constantly removes calcium ions from the solution and shifts the equilibrium to one side only; at low pH values this effect is less marked, the H-ion concentration alone remaining the determining factor.[9]

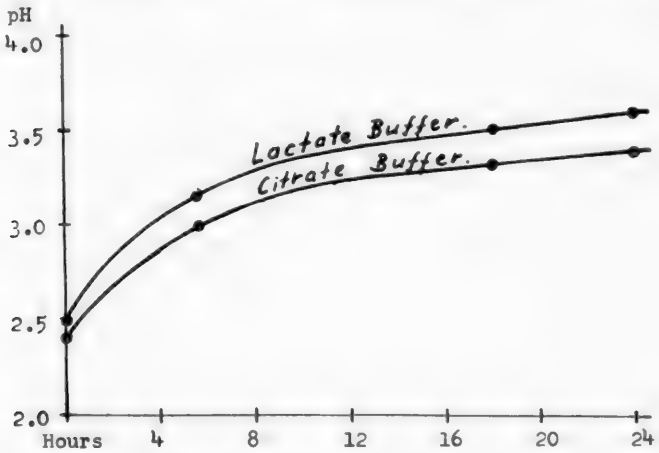

Fig. 9.—Change of pH with time.

Our experiments were carried out at two temperatures, at 20° C. and at 37° C. Though the curves of loss of weight were found to follow the same characteristic trends, they convey an idea how the rate of dissolution increases with rise in temperature. This is of significance, with respect to many diseases accompanied by fever, when the mouth hygiene is often neglected. Evidently more hydrogen ions are exchanged in a warm solution than in one which is at equilibrium relative to a lower temperature.

Though the phenomenology and the actual mechanism of caries should not be considered as constituted solely by decalcification, the concepts evolved from this study may be of significance as they measure a few isolated factors and give us in terms of weight losses a measure of the order of possible injury of the tooth structure from acid decalcification. Yet, back of the problem of acidity is the still greater problem of clinical caries with systemic factors not yet determined and bacterial antagonism playing undoubtedly a major role in the complex picture.

In the planning and preparation of this paper the author enjoyed the cooperation of Dr. A. Katchalski, Department of Macromolecular Chemistry, Hebrew University, for which

his gratitude is here expressed. His thanks are also due to Dr. J. Jordan, Department of Inorganic and Analytical Chemistry, Hebrew University, for his assistance in the beginning stages of this investigation.

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SOME PHYSICAL PROBLEMS INVOLVED IN THE APPLICATION OF SOLUTIONS TO TEETH FOR THE PURPOSE OF REDUCING DENTAL CARIES

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INTRODUCTION

IN VIEW of the common clinical practices of using bacteriostatic mouthwashes and topically applied solutions in attempts to reduce dental caries, it appears worth while to examine certain theoretical considerations limiting the effectiveness of such practices.

There are two factors opposing the penetration of the active molecules or ions of topically applied solutions into contact and similar areas: (1) the presence (in pits, grooves, and contact areas) of saliva or air which must be displaced if the solution is to wet these areas completely and (2) the fact that diffusion of substances in solutions into small spaces is slowed down by the mechanism discussed by Rashevsky[1] and Besic.[2]

The following experiments illustrate the operation of these factors.

EXPERIMENTAL PROCEDURES

1. A highly polished steel ball ¾ inch in diameter was immersed in a 1.0 per cent solution of copper sulfate and immediately brought into contact with another similar ball. In 5 minutes the balls were removed from the solution and examined (Fig. 1). The arrow (*X*) shows an area surrounding the actual contact point devoid of any appreciable corrosion. The dark area is heavily corroded by the $CuSO_4$ solution. This experiment shows, as has been previously explained by Besic,[2] that the original amount of $CuSO_4$ present in the contact area when the two balls were brought together in the solution was not sufficient to produce noticeable corrosion since the surface area of the contact regions of both balls is great compared with the small volume of $CuSO_4$ solution in the narrow "contact" space. It is evident that additional copper and sulfate ions did not diffuse rapidly enough from the surrounding solution into the contact area to produce a decided reaction.

2. Two steel balls ¾ inch in diameter were placed in contact with each other in air. Several drops of a 5.0 per cent solution of $CuSO_4$ were placed directly over the contact region. In 5 minutes the contact regions were examined (Fig. 2). A lack of corrosion is seen at *X*, the actual contact point, and in the surrounding region which acquired an elliptical form because of gravitational forces acting upon the volume of solution trapped between the balls. This elliptical form of the uncorroded area occurs only when air is trapped in the contact region. Elimination of the air in that region produces a circular uncorroded

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region as is seen in Fig. 3. The presence of air in a contact region of course prevents any reaction from occurring in that area. The dark area (*Y*, Fig. 2) is corroded by the solution that surrounded the zone occupied by air.

3. Two balls were again placed in contact with each other in air. Immediately after several drops of a 5.0 per cent solution of $CuSO_4$ were placed directly over the contact region, a piece of dental floss was carried through the contact point once downward and then once upward to eliminate the trapped air. After the solution was allowed to remain untouched for 5 minutes the balls were separated and examined. The contact point (*X*, Fig. 3) and the surrounding area remain uncorroded. The dark region surrounding the clear area is corroded by the solution which was at a distance sufficiently remote from the contact point to permit unhindered diffusion of active ions from the bulk of the liquid. The elliptical form of the corroded region is again due to the force of gravity acting upon the volume of the solution between the balls. Therefore,

Fig. 1.

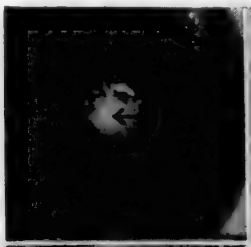

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 1.—Portion of ball corroded by 1.0 per cent solution of $CuSO_4$. The contact point (*X*) and surrounding region is uncorroded. Heavy corrosion of the remaining surface is indicated by *Y*.

Fig. 2.—Portion of steel ball showing result of dropping $CuSO_4$ solution over contact area. Contact point (*X*) and surrounding clear area uncorroded because of trapped air during experiment. Corroded area indicated by *Y*. Uncorroded and corroded zones assumed elliptical form because of gravitational force on volume of solution held between balls.

Fig. 3.—Air bubble removed by dental ligature but uncorroded zone persists. Contact point designated by *X*. Corroded area indicated by *Y*.

Fig. 4.—Clear area in contact region (center of whole dark zone) practically obliterated by constant agitation of $CuSO_4$ solution with thin metal strip.

it is evident that after trapped air was removed by dental floss there was no noticeable corrosion in the contact area after a period of 5 minutes. Evidently there were insufficient $CuSO_4$ ions present in solution in that zone to produce a detectable reaction. Likewise, sufficient ions were unable to diffuse into the area from the surrounding bulk solution so as to produce a pronounced effect.

4. Two balls were again placed in contact with each other in air. Immediately after several drops of a 5.0 per cent solution of $CuSO_4$ were placed

directly over the contact region, a thin steel metal strip (.001 inch thick) was continuously drawn to and fro (from left to right and right to left) through the contact area for 5 minutes. The uncorroded zone is practically obliterated as is shown in Fig. 4.

5. A 5.0 per cent solution of $CuSO_4$ was placed in a dental spray bottle. The nozzle of the spray bottle was directed over the contact point formed by two approximating ¾ inch steel balls and the full force (8 to 10 lbs. per sq. inch) of compressed air was used to spray the area for 2 minutes. The result is shown in Fig. 5. The spray of $CuSO_4$ from above downward caused corrosion of the balls right to the point of contact. From that point the spray formed a pathway around and some distance below the contact point causing corrosion where it passed over the ball surface. A large uncorroded area persisted, however, below the contact area as shown by *Y* in Fig. 5. Presumably trapped air occupied the uncorroded area during the experiment.

6. Two steel balls were placed in contact with each other and a piece of paraffin was molded between the balls below the contact point in order to simulate more closely the physical relationship that is created by the presence of the septal tissue in the interproximal area. The wax was luted to the balls in several places but did not touch the contact point. Roughly, it just filled the area below the point of contact. A 5.0 per cent solution of $CuSO_4$ was sprayed directly at the contact point from above for 2 minutes. The actual contact point is shown at *X* in Fig. 6 with a surrounding uncorroded area. The dark

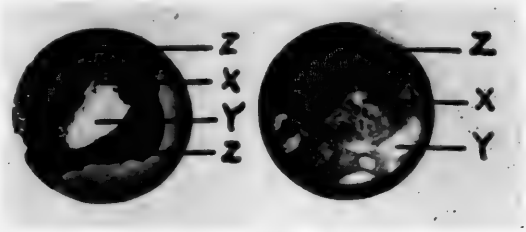

Fig. 5. Fig. 6.

Fig. 5.—Effect of spraying contact region with $CuSO_4$. *X*, Contact point; *Y*, uncorroded area below contact point; *Z*, corroded area.

Fig. 6.—Effect of placing wax rim below contact point between balls and spraying region with $CuSO_4$. *X*, Contact point and surrounding uncorroded area which is many times larger than the actual contact point. *Y*, an area where wax was luted to ball; *Z*, corroded area.

region (*Z*) was heavily corroded. Other clear spots (*Y*) on the ball were caused by luting the wax to the surface in those areas. Thus the introduction of a wax body between the balls diverted the spray of $CuSO_4$ closer to the contact point but a small uncorroded area still persisted after such treatment. When the spray was directed toward the contact point from a lateral direction, larger uncorroded areas were formed on the ball surfaces extending from the point of contact outward toward the opposite lateral surfaces.

Recapitulating the foregoing, one may conclude that air is trapped in the contact area between two spheres when several drops of a 5.0 per cent solution

of $CuSO_4$ are placed directly between the balls. By passing dental floss through the area one obliterates the air bubble but practically no reaction takes place in the critical region because of the relatively small volume of $CuSO_4$ solution present in this narrow space compared to the large area composed by the surfaces of the two balls in contact with each other. Also a hindrance in diffusion of additional ions from the surrounding solution into the region of the contact point and immediate vicinity nullifies any further corrosion in 5 or more minutes. Constant agitation of the solution in this area with a thin metal strip practically eliminated the uncorroded zone. The use of compressed air for the purpose of spraying contact areas with a 5.0 per cent solution of $CuSO_4$ did not eliminate the clear area around the point of contact although the introduction of a substance to fill some of the space between the balls (below, or opposite to the direction from which the spray was directed) reduced the size of the uncorroded area.

The problem becomes more complex in dealing with deep pits and grooves which may be likened to small capillary tubes sealed at one end. The following experiments (Experiments 7-9) show some of the problems encountered when one attempts to place effectively the active ions or molecules of a solution into the deepest parts of small capillary tubes.

7. Fig. 7 shows a capillary tube 0.2 mm. in diameter near the closed end, filled with water to the level indicated by the arrow, *X*. A 0.1 per cent solution of methylene blue was layered over the water with the aid of a fine wire. Diffusion of methylene blue from above downward was very slow and in four hours progressed to the level indicated by arrow, *Y*. A similar experiment employing potassium ferrocyanide instead of methylene blue showed some diffusion into the water toward the apex of the tube. Other substances with high molecnlar weight did likewise (gravitational force). This, however, did not occur when the capillary tube was inverted, placing the water above the heavier solution. Thus, if the smallest trace of saliva remained in the deepest part of a fine dental pit or groove, diffusion of any solution into it would not occur in several minutes unless aided materially by gravitational forces. This would apply in capillaries of the size mentioned (0.2 mm.). The difficulty may be even greater in finer channels.

8. Assuming that saliva can be removed from the bottom of a deep pit or groove, we are confronted with a more difficult problem since air now occupies the bottom of the capillary. A closed capillary tube is represented in Fig. 8. The dark methylene blue solution could be carried into the tube only as far as a fine wire could pass. The remaining clear region in the tube is filled with trapped air.

9. The dye solution (Fig. 9) was brought successfully to the deepest part of the capillary tube by prolonged jarring of the vessel with a solid object. The treatment of deep pits and grooves in teeth with solutions by a similar technic would be impractical in vivo.

DISCUSSION

These data suggest that our present methods of applying chemical agents to teeth to reduce dental caries are not completely satisfactory. The recent employment of topically applied solutions for the purpose of reducing dental caries demands a critical survey of some other pertinent facts.

Knutson and Armstrong's report[3] and subsequent ones by other writers claim a maximum of 40 per cent reduction in dental caries in population groups during the first year after treatment with sodium fluoride. None of these reports indicates which class of lesions is affected most by fluoride treatment or if all classes are equally reduced. Statistics showing which of the three classes of lesions is most reduced would be interesting as related to the findings

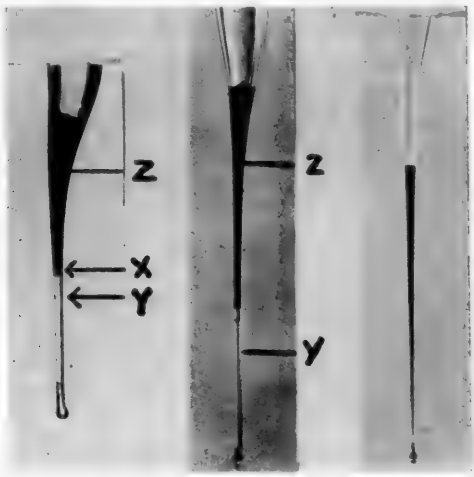

Fig. 7. Fig. 8. Fig. 9.

Fig. 7.—H_2O from tip of capillary tube to level *X*. *Z*, Methylene blue solution. Diffusion of methylene blue from *X* to *Y* in 4 hours.
Fig. 8.—*Z*, Methylene blue solution; *Y*, trapped air.
Fig. 9.—Methylene blue solution carried to bottom of capillary tube by jarring.

in this paper. Arnold, Dean, and Singleton[4] in an apparently well-conducted study report no reduction in caries after a single application of a very dilute acidulated solution of sodium fluoride. Roberts, Bibby, and Wellock[5] report that an acidulated fluoride mouthwash actually increased the caries rate when compared with an acidulated nonfluorinated mouthwash. Krasnow[6] in a critical statistical review of the literature stated: "Relative percentage variations in the rates per cent of treatment versus non-treatment characteristically magnify the rates per cent." It is obvious that at least some controversy over present clinical statistics does exist.

The findings in this paper apply not only to topical fluoride applications but to the application of any other chemicals.

Dental caries is initiated at the bottom of deep pits and grooves, in the neighborhood of contact areas, and in some gingival third and other smooth

surface areas that have certain physical relationships with soft tissues. Since the lips, cheeks, and tongue may be retracted easily, such (gingival third) areas can be easily approached and perhaps effectively treated. If there are any benefits to be derived from the topical applications of solutions to teeth, they should be manifested particularly in the buccal and labial gingival third areas. Clinical statistics are not available to verify this assertion.

Chemical agents, that are employed to reduce dental caries in one way or another, must be applied in such a manner that they reach effectively the precise regions where carious lesions begin. That this might be accomplished by present methods in gingival third areas is not denied, but the difficulties active ions encounter in reaching the enamel surface in other regions are obvious.

CONCLUSIONS

1. It is difficult to introduce solutions into contact areas, pits, and grooves.

2. A hindrance in diffusion of active molecules and ions into narrow spaces from the bulk solution (outside of these spaces) prevents the maintenance of a satisfactory concentration of these active particles.

3. Solutions applied to teeth for the purpose of reducing dental caries should be most effective in gingival third areas with our present methods of application.

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THE EVANSTON DENTAL CARIES STUDY

V. The Fluorine Content of Saliva and Its Relationship to (A) Oral Lactobacillus Counts and (B) the Prevalence of Dental Caries

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McCLURE[5] reported in 1941 that he found 0.08 parts per million of fluorine (F) in 200 pooled specimens of salivas, obtained from 12- to 14-year-old white children in Evanston. He also stated that no conclusions were justified at the time with regard to the significance of the quantity of fluorine which is normally present in saliva. It has also been reported by Cox[2] and McClure[6] that (A) the fluorine content of human saliva is of the order of only 0.10 ppm and (B) that it is not significantly different in the salivas of children where the fluorine content of the water is below 1.80 ppm. As early as 1936 White and Bunting[7] found no apparent relationship between chemical constituents (excluding fluorine) of the saliva and dental caries.

Taking into consideration the work just reviewed, and the lack of conclusive evidence, we decided to conduct our own investigation of the specimens of saliva collected from the school children in Evanston each week. In this report we are primarily concerned with the following: (1) the analysis of saliva to determine its fluorine content, and (2) the relationship between the fluorine content of saliva (A) to the oral lactobacillus count and (B) to the prevalence of dental caries.

APPARATUS AND TECHNIC

Stimulated salivary specimens are collected from 10 per cent of the children in the Dental Caries Study. These collections are made on Monday of each school week. A corked bottle one and one half by two inches is used to collect 5 to 8 c.c. of saliva and the time required to secrete that amount is recorded. The specimens are collected prior to the dental examination and the taking of x-ray films. The specimen bottles are labeled and placed in an iced thermos jug to retard bacterial growth and at the close of the school day are brought to the laboratory where they are plated the same day.

A medium of tomato juice, agar, yeast extract, peptonized milk, and peptone is used and a pH of 5.2 is maintained. A Beckman pH meter is frequently used to check the pH of the medium. For the purpose of comparison, four plates are inoculated from each salivary specimen. Two plates are inoculated with 0.1 c.c. of a 1 to 10 dilution and two more plates are inoculated with 0.1 c.c. of a 1 to 100 dilution. Approximately 0.4 to 0.6 c.c. of normal saline is used on

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each plate to maintain an even distribution of the inoculum. An incubator of ample size is employed to hold all the plates which are incubated for 72 hours at 37° Centegrade.

A smear from each of the various types of colonies present is made so as to identify the microorganisms by using the Hadley and Bunting classification.[3] The purpose of making a smear is to insure that the other aciduric organisms which are found in the mouth and grow in a medium of this pH (5.2) are recognized and differentiated from the oral lactobacillus. The average of the two plates of the 1 to 10 dilution is taken as the count for that specimen. All counts are done by the same individual.

The fluorine content of the saliva is determined by the method of Willard and Winter,[8] using the modifications of the Association of Official Agricultural Chemists[1]. Magnesium acetate is employed as the fluorine fixative. The stills are of Pyrex glass with all glass connections as recommended by the Associate Referee of the Food and Drug Administration, Washington, D. C. The flasks are a Claissen type distilling flask. All precautions are taken to maintain a minimum distillation blank, including the use of platinum dishes when ashing the saliva.

In previous reports it has been shown that 100 c.c. samples of saliva were used for an analysis. Before proceeding with this work, the authors collected 150 c.c. samples of saliva in the laboratory, divided them into 100 and 50 c.c. samples, respectively, and found only a slight, but negligible, difference in the fluorine content of the two portions of the samples. With this information it was decided to use approximately 50 c.c. of saliva for each determination.

The salivas are pooled according to their respective oral lactobacillus counts, that is, all negative counts are separated from the positive counts and an average of five specimens are pooled for each analysis. The salivary specimens from the 6-7-8-year age group are not pooled with specimens from the 12-13-14-year age group.

The time interval of exposure to the sodium fluoride, which has been present in the Evanston water supply since February, 1947, varied in the two groups studied. Inasmuch as the two different age groups were not examined simultaneously, the 6-7-8-year age group was exposed to the fluorinated water from 20 to 25 months and the 12-13-14-year age group from 25 to 29 months. This investigation was carried on for one school year, and data were collected from September, 1948, to June, 1949.

RESULTS AND DISCUSSION

Table I shows the fluorine content of saliva and its relationship to the oral lactobacillus count. These data are for the 6-7-8-year age group and is for the period of time from September, 1948, to February, 1949. The 35 fluorine determinations represent 188 pooled salivary specimens, which is approximately five pooled specimens per analysis. For the sake of clarity, the figures given for the parts per million of fluorine are not listed in the order in which the analyses were made in the laboratory, but have been arranged in an ascending

order. From this table it will be seen that the fluorine content of the salivas varies from 0.14 ppm to 0.35 ppm with an average of 0.25 ppm and a mean of 0.27 ppm. The oral lactobacillus counts listed are averages of the specimens which have been pooled for each fluorine analysis. The averaged counts, which are of lactobacillus and do not include other aciduric organisms,[4] ranged from negative to 39,800 lactobacilli per cubic centimeter. This Table shows that there is no apparent relationship between the fluorine content of saliva and the oral lactobacillus count.

TABLE I

RELATIONSHIP BETWEEN FLUORINE CONTENT OF SALIVA AND THE ORAL LACTOBACILLUS COUNTS IN THE 6-7-8-YEAR AGE GROUP

PPM FLUORINE	LACTOBACILLUS COUNT* 1 × 10 DIL.	PPM FLUORINE	LACTOBACILLUS COUNT* 1 × 10 DIL.
0.14	Neg.	0.27	39,800
0.16	4,180	0.28	Neg.
0.16	658	0.29	Neg.
0.16	25,510	0.29	Neg.
0.17	Neg.	0.29	1,900
0.18	13,940	0.29	Neg.
0.18	2,690	0.29	20,230
0.19	21,480	0.30	4,320
0.20	10,080	0.31	36,020
0.21	Neg.	0.31	Neg.
0.21	Neg.	0.31	37,490
0.21	4,320	0.32	Neg.
0.23	14,900	0.32	18,550
0.25	Neg.	0.32	3,930
0.26	13,260	0.33	1,045
0.26	12,900	0.34	1,550
0.27	Neg.	0.35	14,500
0.27	Neg.		

*Average count of specimens pooled for each analysis.

In Table II the fluorine content of the saliva is shown in relationship to the prevalence of dental caries in the 6-7-8-year age group. For this investigation the lesions of dental caries has been divided into four groups: (1) untreated carious surfaces found clinically, (2) carious surfaces found only by roentgenogram, (3) teeth extracted due to dental caries, and (4) restored surfaces. All of the figures given for the carious surfaces found clinically, carious surfaces found by roentgenogram, the number of extractions, and the restored surfaces are averages for the children whose salivas were pooled for each fluorine analysis. In reviewing Table II, it is seen that the untreated carious surfaces found clinically varied from 0.00 to 19.50; the carious surfaces found by roentgenogram varied from 0.00 to 4.40; the number of extractions varied from 0.00 to 1.75, and the restored surfaces from 0.00 to 13.80. Examination of this Table shows that there is no apparent relationship between the fluorine content of the saliva and the prevalence of dental caries.

Table III shows the fluorine content of saliva and its relationship to the oral lactobacillus count for children in the 12-13-14-year age group. These data are for the period of time from March, 1949, to June, 1949. For this Table the parts per million of fluorine are also listed in ascending order and the 28 fluorine determinations represent 124 pooled specimens, which is approxi-

TABLE II

RELATIONSHIP BETWEEN FLUORINE CONTENT OF SALIVA AND THE PREVALENCE OF DENTAL CARIES IN THE 6-7-8-YEAR AGE GROUP

PPM FLUORINE	UNTREATED CARIOUS SURFACES*		TREATED CARIOUS SURFACES*	
	CLINICALLY	ROENTGENOGRAM	FILLINGS	EXTRACTIONS
0.14	0.00	0.00	0.00	0.00
0.16	6.80	2.80	8.40	0.00
0.16	3.80	2.40	5.60	0.60
0.16	5.33	3.66	11.33	0.00
0.17	0.60	1.20	1.40	0.00
0.18	7.20	3.50	3.33	0.33
0.18	7.60	1.40	8.20	1.00
0.19	3.20	4.40	8.60	0.20
0.20	7.40	3.40	7.00	0.10
0.21	2.00	3.10	2.60	0.00
0.21	1.25	1.75	7.50	0.00
0.21	3.40	1.40	13.80	0.80
0.23	3.00	2.33	6.66	0.00
0.25	1.80	2.20	4.80	0.00
0.26	9.50	2.75	11.50	0.00
0.26	9.50	2.50	10.70	0.00
0.27	5.00	2.25	6.25	0.00
0.27	0.50	1.25	0.00	0.00
0.27	12.00	2.60	4.00	0.20
0.28	1.00	3.33	3.33	0.00
0.29	1.00	2.25	2.10	0.00
0.29	2.66	1.00	1.33	0.00
0.29	19.50	3.50	6.75	0.50
0.29	5.00	0.50	6.60	0.00
0.29	7.60	3.20	7.80	0.00
0.30	1.00	2.33	6.33	0.00
0.31	3.80	1.66	7.33	0.00
0.31	6.80	2.20	1.00	0.00
0.31	10.50	0.75	5.00	1.75
0.32	2.00	1.40	2.00	0.00
0.32	11.00	4.00	6.66	0.00
0.32	3.60	2.20	5.10	0.20
0.33	2.20	1.20	3.00	0.10
0.34	1.60	1.60	1.20	0.00
0.35	14.50	3.50	7.50	0.75

*Averages of specimens pooled for each analysis.

TABLE III

RELATIONSHIP BETWEEN FLUORINE CONTENT OF SALIVA AND THE ORAL LACTOBACILLUS COUNTS IN THE 12-13-14-YEAR AGE GROUP

PPM FLUORINE	LACTOBACILLUS COUNT* 1 × 10 DIL.	PPM FLUORINE	LACTOBACILLUS COUNT* 1 × 10 DIL.
0.11	Neg.	0.20	5,930
0.14	Neg.	0.20	10,900
0.14	92,320	0.20	23,300
0.15	Neg.	0.21	600
0.16	22,375	0.21	33,450
0.17	Neg.	0.22	Neg.
0.17	Neg.	0.23	Neg.
0.18	Neg.	0.23	430
0.18	5,470	0.23	7,980
0.18	16,280	0.25	2,760
0.18	18,930	0.25	11,570
0.18	25,780	0.26	400
0.19	1,060	0.26	34,280
0.20	4,010	0.29	2,060

*Average count of specimens pooled for each analysis.

mately five pooled specimens per analysis. From this Table it is to be seen that the fluorine content of the salivas varied from 0.11 ppm to 0.29 ppm with an average of 0.20 ppm and a mean of 0.20 ppm. The averaged oral lactobacillus counts ranged from negative to 92,320 lactobacilli per cubic centimeter. Table III also shows that there is no apparent relationship between the fluorine content of saliva and the oral lactobacillus count.

TABLE IV

RELATIONSHIP BETWEEN FLUORINE CONTENT OF SALIVA AND THE PREVALENCE OF DENTAL CARIES IN THE 12-13-14-YEAR AGE GROUP

PPM FLUORINE	UNTREATED CARIOUS SURFACES*		TREATED CARIOUS SURFACES*	
	CLINICALLY	ROENTGENOGRAM	FILLINGS	EXTRACTIONS
0.11	4.25	2.50	7.75	0.00
0.14	8.33	4.33	7.66	0.00
0.14	11.33	5.00	16.60	0.20
0.15	17.33	6.00	1.33	0.33
0.16	6.00	3.00	6.50	0.00
0.17	1.80	0.80	9.40	0.00
0.17	4.25	2.75	8.00	0.00
0.18	3.33	5.66	8.66	0.00
0.18	6.00	4.25	5.25	0.00
0.18	14.20	4.00	9.33	0.20
0.18	10.33	4.66	8.33	0.00
0.18	14.20	2.50	12.20	0.20
0.19	8.00	4.00	8.00	0.00
0.20	9.80	8.40	12.00	0.00
0.20	6.60	4.40	10.60	0.00
0.20	9.50	4.66	9.66	0.20
0.20	18.25	4.00	1.25	0.25
0.21	5.60	7.00	17.60	0.00
0.21	8.00	6.00	9.75	0.00
0.22	4.80	3.20	5.40	0.00
0.23	6.00	2.50	7.75	0.00
0.23	14.00	5.75	1.25	0.25
0.23	5.00	1.66	10.10	0.00
0.25	6.00	1.75	12.00	0.25
0.25	9.40	2.40	7.40	0.60
0.26	6.80	6.00	7.40	0.00
0.26	12.40	3.20	8.20	0.40
0.29	4.40	2.40	6.80	0.00

*Averages of specimens pooled for each analysis.

In Table IV the relationship between the fluorine content of saliva and the prevalence of dental caries in the 12-13-14-year age group is shown. In this Table the number of carious lesions is reported on the same basis as it is in Table II. This Table shows that the carious surfaces found clinically varied from 1.80 to 18.25; the carious surfaces found by roentgenogram from 0.80 to 8.40; the number of extractions from 0.00 to 0.60, and the restored surfaces from 1.25 to 16.60. Table IV also shows that there is no apparent relationship between the fluorine content of saliva and the number of carious lesions observed.

SUMMARY

1. As shown in Table V, the average, the mean, the maximum, and minimum parts per million of fluorine in saliva were lower in the 12-13-14-year age group than in the 6-7-8-year age group. The average for the two groups being 0.20 ppm and 0.25 ppm of fluorine.

TABLE V

COMPARISON OF DATA FOR THE 6-7-8 AND 12-13-14-YEAR AGE GROUPS STUDIED

AGE GROUP	PPM F IN SALIVA		PPM F IN SALIVA	MAXIMUM AND MINIMUM FOUND	
	AVERAGE	MEAN		AV. LACTOBACILLUS COUNT	TOTAL CARIOUS SURFACES TREATED AND UNTREATED
6-7-8	0.25	0.27	0.14-0.35	Neg.-39,800	0.00-30.25
12-13-14	0.20	0.20	0.11-0.29	Neg.-92,320	12.00-33.10

TABLE VI

RELATIONSHIP OF THE ORAL LACTOBACILLUS COUNT AND TOTAL DENTAL CARIES EXPERIENCE TO THE FLUORINE CONTENT OF SALIVA IN THE 6-7-8-YEAR AGE GROUP

LACTOBACILLUS COUNT* 1 × 10 DIL.	DENTAL CARIES EXPERIENCE		PPM F IN SALIVA
	UNTREATED CARIOUS SURFACES*	RESTORED SURFACES*	
Neg.	0.00	0.00	0.14
Neg.	1.80	1.40	0.17
Neg.	5.10	2.60	0.21
Neg.	3.00	7.50	0.21
Neg.	4.00	4.80	0.25
Neg.	1.75	0.00	0.27
Neg.	4.33	3.33	0.28
Neg.	3.25	2.10	0.29
Neg.	3.66	1.33	0.29
Neg.	5.50	6.60	0.29
Neg.	9.00	1.00	0.31
Neg.	3.43	2.00	0.32
658	6.20	6.20	0.16
1,045	3.44	3.10	0.33
1,550	3.20	1.20	0.34
1,890	23.00	7.25	0.29
2,690	10.67	3.66	0.18
3,930	5.80	5.30	0.32
4,180	9.60	8.40	0.16
4,320	7.80	14.60	0.21
4,320	3.33	6.33	0.30
10,080	10.76	7.10	0.20
12,900	12.00	10.70	0.26
13,260	12.25	11.50	0.26
13,940	9.00	9.20	0.18
14,500	17.75	8.25	0.35
14,900	5.33	6.66	0.23
18,550	15.50	6.66	0.32
20,230	10.80	7.80	0.29
21,480	7.60	8.80	0.19
25,510	9.00	11.33	0.16
36,020	5.50	7.33	0.31
37,490	11.25	6.75	0.31
39,800	14.60	4.20	0.27

*Averages of pooled specimens.

2. Tables VI and VII have been prepared to show the relationships of the average oral lactobacillus counts and the average total dental caries experience to the parts per million of fluorine in the saliva. These tables are a regrouping of the previous tables discussed. All of the data which we have presented shows that there is no apparent relationship between the fluorine content of saliva and (A) the oral lactobacillus count or (B) the prevalence of dental caries.

TABLE VII

RELATIONSHIP OF THE ORAL BACTOBACILLUS COUNT AND TOTAL DENTAL CARIES EXPERIENCE TO THE FLUORINE CONTENT OF SALIVA IN THE 12-13-14-YEAR AGE GROUP

LACTOBACILLUS COUNT* 1 × 10 DIL.	DENTAL CARIES EXPERIENCE		PPM F IN SALIVA
	UNTREATED CARIOUS SURFACES*	RESTORED SURFACES*	
Neg.	6.75	7.75	0.11
Neg.	12.66	7.66	0.14
Neg.	23.33	1.66	0.15
Neg.	7.00	8.00	0.17
Neg.	2.60	9.40	0.17
Neg.	9.00	8.66	0.18
Neg.	8.00	5.40	0.22
Neg.	8.50	7.75	0.23
400	12.80	7.40	0.26
430	19.75	1.50	0.23
600	12.60	17.60	0.21
1,060	12.00	8.00	0.19
2,060	7.00	6.80	0.29
2,760	7.75	12.25	0.25
4,010	18.25	12.00	0.20
5,470	10.25	5.25	0.18
5,930	11.00	10.60	0.20
7,980	6.66	10.10	0.23
10,900	14.20	9.80	0.20
11,570	11.80	8.30	0.25
16,280	18.20	9.50	0.18
18,930	15.00	8.33	0.18
22,375	9.00	6.50	0.16
23,300	22.25	1.50	0.20
25,780	16.70	12.40	0.18
33,450	14.00	9.75	0.21
34,280	15.60	8.60	0.26
92,320	16.33	16.80	0.14

*Averages of pooled specimens.

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EVALUATION OF THE COLORIMETRIC (SNYDER) TEST

I. Comparison of Positive Color Reactions With the Lactobacillus Counts of Respective Specimens of Saliva Routinely Submitted for Culture

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A SIMPLE colorimetric test for the estimation of numbers of lactobacilli in the saliva was described in 1940 by Snyder.[1] Its accuracy as a laboratory test for the estimation of caries activity was shown to be equal at least to the standard "lactobacillus count" in a study of children whose clinical status was determined by periodic dental and roentgenologic examination.[2, 3] The simplicity of this test permits its ready adaptation to the dentist's office since it requires only a few pipettes, tubes of culture media, and a small incubator. Despite this advantage over the strictly laboratory requirements of the lactobacillus count, there have been only scattered references to the possible usefulness of the colorimetric test, commonly referred to as the Snyder test, as a clinical tool.[4, 5] Because of numerous communications concerning its application, it was thought of interest to submit a report comparing a series of colorimetric tests and the lactobacillus counts on respective specimens of saliva received at the Caries Diagnostic Laboratory Service.*

PROCEDURE

The material consisted of specimens of saliva submitted for lactobacillus counts, which were performed in the manner described by Hadley[6] with the exception that phenol red glucose broth (pH 7.4) was substituted for the acid glucose broth (pH 5.0). This modification was made for the purpose of determining alkalinization of the saliva-glucose broth cultures after initial acidity; these results have been published elsewhere.[7] At the time of plating the saliva on tomato juice peptone agar, 0.2 c.c. of the same specimen was transferred to a tube of melted bromeresol green dextrose agar (pH 5.)† held at 50° C., and then mixed and allowed to solidify. Color changes were graded in terms of 0, +, ++, +++, ++++ compared with an uninoculated control tube and recorded daily for three days. To those unacquainted with this test, it should be said that there is no difficulty in reading the extremes (0 or ++++) or slight deviations from the bluish-green of the negative to the yellow of maximal change; it is the decision whether green is no longer the dominant color (++) where individual variation will be greatest. This problem was answered in the present study by recording all color reactions significantly different from the control as positive for that date and specimen. The correlation of positive color change with lactobacillus counts in respective specimens of saliva is given in Table I.

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*Established at the University of Oregon Dental School in cooperation with the State Department of Health and the Children's Bureau.

†Obtainable from Difco Laboratories, Detroit, Mich., either in bulk or tubed for use.

TABLE I

COMPARISON OF POSITIVE COLOR CHANGES IN BROMCRESOL GREEN DEXTROSE AGAR (pH 5.0) INOCULATED WITH 0.2 c.c. SALIVA AND LACTOBACILLUS COUNTS OF RESPECTIVE SPECIMENS OF SALIVA

NO. LACTOBACILLI/C.C. SALIVA	NO. SPEC.	POSITIVE COLOR REACTIONS (HOURS)					
		24		48		72	
		POS.	% POS.	POS.	% POS.	POS.	% POS.
0	348	2	0.6	22	6.3	85	24.5
0-100	59	0	0.0	7	11.9	32	54.3
100-1,000	157	5	3.2	47	30.0	111	70.0
1,000-5,000	203	8	4.0	104	51.7	183	91.2
5,000-10,000	105	6	5.7	69	65.7	99	94.2
10,000-20,000	138	18	13.0	105	76.1	131	95.0
20,000-50,000	264	59	22.3	229	86.8	260	98.5
50,000-100,000	245	72	29.3	201	90.3	243	99.3
100,000--------	497	231	46.4	476	95.5	494	99.3

Analysis of Table I shows that the incidence of positive color change increases steadily with the lactobacillus count and time. The following points are of interest: (a) there is no absolute correlation since there are a few exceptions at the extremes; (b) the only really sharp break in the frequency of positive color tests compared with the lactobacillus count occurs in 24 hours at the 10,000 figure level, and (c) a maximum of 46.6 per cent of color change in 24 hours was recorded with counts greater than 100,000 lactobacilli/c.c. saliva. This last figure is in contrast with the 87.0 per cent previously reported from clinically active children with similar lactobacillus counts.[3] An explanation could be made that the latter figure was obtained with a relatively small number of freshly collected specimens while the former was made with specimens of saliva received through the mails without knowledge of the individual clinical status.

The objective of the colorimetric test is, however, to provide the practicing dentist with a relatively simple test which will permit him to select rapidly the extremes of caries activity. The only real evaluation of the colorimetric test as a useful procedure is by clinical correlation which, in this case, was impossible. This leaves only an indirect comparison by means of the lactobacillus count which it has already been shown to parallel in respect to caries activity on a qualitative basis.[3] Since this relationship has been established, it should be reasonable to compare the data in the present study over larger quantitative ranges than depicted in Table I. This analogy in respect to clinical change is not, however, that easy or clear since the workers in the field are not in harmony on the matter of quantitative figures and degree of clinical activity. There seems to be general agreement that counts of less than 1,000 lactobacilli/ml. saliva are indicative of negative to slight caries activity, but above this level wide differences in opinion occur. Thus, Jay[8] maintains that dietary therapy should be instituted when the lactobacillus count goes above 10,000/c.c. saliva, whereas Robinson[9] states that counts between 1,000 and 50,000 are of doubtful value, and differentiation of those above this limit meaningless for purposes of evaluation of effectiveness of control methods. Our data indicate that in so far as the lactobacillus count is concerned, a definite break in incidence of positive

color tests in 24 hours occurred at the 10,000 figure level. For that reason the data presented in Table I are condensed in Table II in respect to this figure.

TABLE II

COMPARISON OF POSITIVE COLOR CHANGES IN BROMCRESOL GREEN DEXTROSE AGAR (pH 5.0) INOCULATED WITH 0.2 C.C. SALIVA AND LACTOBACILLUS COUNTS IN RESPECTIVE SPECIMENS OF SALIVA DIVIDED IN RESPECT TO 10,000 LACTOBACILLI/C.C. OF SALIVA

NO. LACTOBACILLI/C.C. SALIVA	NO. SPEC.	POSITIVE COLOR REACTIONS (HOURS)					
		24		48		72	
		POS.	% POS.	POS.	% POS.	POS.	% POS.
0-10,000	872	21	2.4	249	28.5	362	41.5
10,000-------	1144	380	33.4	1031	90.0	1128	98.5

Table II has some interesting data. Thus, only 2.4 per cent of specimens containing less than 10,000 lactobacilli/c.c. saliva induced positive color change in 24 hours contrasted with 33.4 per cent of the specimens with counts higher than 10,000 lactobacilli/c.c. of saliva. This is a ratio of 1:17, which is reduced in 48 hours nearly 1:3. It is also worthy to mention that condensation of the data obtained from the University of Michigan study in respect to this level yields an identical figure to the one in the present study carried out in an entirely different geographical area under far different conditions. On the basis that the positive colorimetric test is essentially dependent upon the presence of lactobacilli and the rate of change to some extent upon numbers, and that these organisms serve as an index of caries activity, it would seem possible by analogy to use this test in the following manner: to select in 24 hours those individuals with high lactobacillus counts who are most likely caries active and in 48 hours those with low counts who are generally considered to be slightly caries active or even in the immune state.

One of the side problems to be answered in use of the Snyder test is the influence on acid production by other oral bacteria with special reference to the yeast forms commonly observed in routine cultures of the saliva. Hansen, Fosdick, Wessinger, and Epple[10, 11] have reported a synergistic effect on the total acid produced from glucose by these organisms and lactobacilli, that is, the amount of acid produced by the combination is greater than that yielded by either acting alone. Previous studies[11] showed these yeast forms, which are almost always members of the genus Candida (Monilia), either in pure culture or in combination with oral streptococci and staphylococci were not able to produce detectable amounts of acid in 24 hours under the anaerobic conditions of growth in the bromcresol green dextrose agar (pH 5.0). Since lactobacilli by themselves changed the color in 24 hours, it was not possible to demonstrate that strains of Candida in combination with lactobacilli accelerated the process during the first 24 hours or increased the amount of acid beyond the indicator, bromcresol green, which has a useful range of pH 3.8 to 5.4.

The culture of these organisms in about 25 per cent of specimens of saliva in this study seemed to offer an opportunity to obtain some answer to this question by comparison of color changes and lactobacillus counts of respective specimens of saliva with or without members of the genus Candida. The results are given in Tables III and IV.

TABLE III

COMPARISON OF POSITIVE COLOR REACTIONS IN BROMCRESOL GREEN DEXTROSE AGAR (pH 5.0) INOCULATED WITH 0.2 C.C. SALIVA AND THE LACTOBACILLUS COUNTS OF RESPECTIVE SPECIMENS OF SALIVA CONTAINING YEASTLIKE ORGANISMS (CANDIDA)

NO. LACTOBACILLI/C.C. SALIVA	NO. SPEC.	POSITIVE COLOR REACTIONS (HOURS)					
		24		48		72	
		POS.	% POS.	POS.	% POS.	POS.	% POS.
0	51	0	0.0	6	11.8	21	41.3
0-100	10	0	0.0	0	0.0	4	40.0
100-1,000	34	1	3.0	10	30.0	22	64.7
1,000-10,000	92	4	4.4	53	57.7	76	83.6
10,000-100,000	236	50	21.2	210	88.9	231	97.8
100,000	109	46	42.2	103	94.5	106	97.2
Total	552						

Examination of these tables shows no essential difference in the percentage of positive color tests induced by specimens of saliva with or without yeastlike organisms in 24 and 48 hours, but there is a significant increase ($\chi^2 = 5.06$ at $p = 0.05$) in the percentage of positive color reactions in 72 hours by specimens of saliva without members of the genus Candida and with less than 10,000 lactobacilli/c.c. of saliva. It can be concluded, therefore, that under laboratory conditions these organisms, which are frequently found in the oral cavity, neither increase nor decrease the amount of acid formed by other oral bacteria inoculated into this medium.

TABLE IV

COMPARISON OF POSITIVE COLOR REACTIONS IN BROMCRESOL GREEN DEXTROSE AGAR (pH 5.0) INOCULATED WITH 0.2 C.C. SALIVA AND THE LACTOBACILLUS COUNTS OF RESPECTIVE SPECIMENS OF SALIVA NOT CONTAINING YEASTLIKE ORGANISMS (CANDIDA)

NO. LACTOBACILLI/C.C. SALIVA	NO. SPEC.	POSITIVE COLOR REACTIONS (HOURS)					
		24		48		72	
		POS.	% POS.	POS.	% POS.	POS.	% POS.
0	297	2	0.7	16	5.3	64	21.4
0-100	49	0	0.0	7	14.3	28	57.2
100-1,000	123	4	3.3	37	30.1	89	72.3
1,000-10,000	216	10	4.6	120	55.7	206	95.3
10,000-100,000	411	99	24.1	345	88.9	402	97.8
100,000	386	185	48.0	373	96.7	379	98.0
Total	1582						

DISCUSSION

From the data presented it is obvious that an extremely close correlation exists between negative color change and counts under 10,000 lactobacilli/c.c. of saliva. On the other hand, with counts above 10,000 lactobacilli/c.c. of saliva, color changes in 24 hours, while increasing beyond any limits of chance, are positive in only one-third of the specimens. In 48 hours, however, 90 per cent of these specimens will have caused a positive color change in this medium. On the basis of these results, as well as those of earlier studies, and the generally accepted relationship between lactobacilli and caries activity, the colorimetric test should prove most effective in estimating caries activity as follows:

Positive color change in 24 hours: marked caries activity

Positive color change in 48 hours: moderate (average) caries activity
Negative color change in 48 hours: slight caries activity
Negative color change in 72 hours: negative caries activity

Thus, the periodic testing of individual patients, especially children, by the colorimetric test should provide useful information to the dentist in three ways: first, the patient who remains consistently negative should have little or no clinical change in the next six months; second, the patient who has been negative but now becomes positive in 24 to 48 hours is entering a state of caries activity; and third, the patient in whom a definite delay in onset of positive color change over previous tests is becoming less active than before, a state which may or may not reflect therapy.

The advantages of the colorimetric test over the lactobacillus count are its simplicity and the rapidity with which extremes of clinical activity can be selected. The disadvantage is the necessity of daily observation whereas a tomato juice agar plate can be placed in the incubator for three or four days and an extra one makes no difference. Jay[13] has also raised this objection to the Snyder test in field surveys where the investigators may be miles from the incubator on the succeeding day. As far as office practice is concerned, however, this factor is negligible if any care is made in planning for the next 48 hours, a time by which the information desired can be obtained.

The interpretation of the Snyder test brings up another problem, the matter of positive or negative color change irrespective of lactobacilli/c.c. or with special reference to the arbitrary limit of 10,000 lactobacilli/c.c. of saliva. A positive color reaction reflects acid production by those organisms introduced into the medium by the inoculum of saliva. It is obvious that the test is not necessarily specific for any one organism, but it is known that with pure cultures of oral staphylococci, streptococci, Candida, and lactobacilli acting either alone or in combination with each other only the lactobacilli produce positive reactions in 24 hours. However, if one accepts decalcification of the enamel as prima facie evidence of acid production, it is in one sense irrelevant which and how many of a single species or combination will accomplish this result. Our data would support the lactobacillus etiology of dental caries but only qualitatively. Quantitatively, the lactobacillus count is open to many objections not only in respect to its accuracy and duplicability[14] but also to the recognition of cases with high counts and low caries activity and those with low counts and definite caries activity. Once again, it is suggested that the rate of acid production may well represent a more accurate index of caries activity than the lactobacillus count. With the development of an adequately controlled clinical program (Child Development Study) in this school, it will become possible to evaluate the Snyder test more fully in regard to clinical change. In the meantime, it is hoped that others may be able to test this hypothesis and thereby more sharply define the value of this simple test.

SUMMARY

Comparison of data obtained by the Snyder test with lactobacillus counts of respective specimens of saliva showed the following relationships:

1. With less than 10,000 lactobacilli/c.c. saliva, 98 per cent of specimens failed to produce a positive color reaction in 24 hours and 71.5 per cent in 48 hours.

2. With more than 10,000 lactobacilli/c.c. saliva, 33.4 per cent of specimens induced positive color tests in 24 hours and 90 per cent in 48 hours.

A practical interpretation of these results for possible office use of the Snyder test is offered.

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ON THE OCCURRENCE OF THE LACTATE FERMENTING ANAEROBE, MICROCOCCUS LACTILYTICUS, IN HUMAN SALIVA

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THE microorganism now known as *Micrococcus lactilyticus** (Foubert and Douglas, 1948a), was first observed and partially described by Lewkowicz (1901) who isolated one strain from the mouth of an infant. The subsequent work of Hall and Howitt (1925) on the anaerobic flora of the mouth added much to our knowledge of this microorganism, and indicated that it was present in large numbers in saliva. However, the methods used by these workers were not suitable for accurately determining the numbers of these bacteria in saliva. This problem was greatly simplified when it was found that *M. lactilyticus* carried out an active fermentation of lactate (Foubert and Douglas, 1948a), a property which is rare among microorganisms, and which made possible the design of more specific cultural methods for determining its incidence in human saliva.

As most treatises (Wilson and Miles, 1946; Appleton, 1944) which deal with the normal flora of the oral cavity either fail entirely to mention the presence of *M. lactilyticus* or at most give it but scanty attention, it seemed desirable to publish our findings on the occurrence of this bacterium in saliva. Since our results show that *M. lactilyticus* is quantitatively one of the most important of the bacteria present in saliva, this fact is bound to be of significance to those interested in the microbial ecology of the oral cavity.

METHODS

Two or three ml. of saliva were collected aseptically from students and colleagues in the laboratory. Serial dilutions were prepared in sterile tap water and plated on Mediums 1 and 2. Colony counts were made after the plates had been incubated anaerobically (95 per cent H_2, 5 per cent Co_2) at 37° C. for 48 hours. Medium 1 consisted of 0.5 per cent Difco yeast extract, 1.0 per cent sodium lactate, and 1.5 per cent agar. Medium 2 contained 2.0 per cent Difco peptone, 0.5 per cent Difco yeast extract, 1.0 per cent glucose, and 1.5 per cent agar. Both media were adjusted to pH 7.0.

M. lactilyticus develops readily on Medium 1, the colonies, which are compact and lens shaped, attaining a diameter of approximately 1.0 mm. in 48 hours. Streptococci also develops anaerobically on this medium but since the

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*Other names applied to this organism in the past have been *Micrococcus gazogenes alkalescens anerobius* (Lewkowicz, 1901), *M. gazogenes* (Hall and Howitt, 1925), and *Veillonella gazogenes* (Prevot, 1933).

colonies rarely exceed 0.1 mm. in diameter they may be easily distinguished from *M. lactilyticus*. Colonies of *M. lactilyticus* may also be identified by the gram reaction, which is negative at this stage, and by the formation of abundant gas within 24 hours in subcultures made by stabbing freshly exhausted deeps of Medium 1.

TABLE I

THE OCCURRENCE OF M. LACTILYTICUS IN SALIVA

SUBJECT	MILLION PER ML.	
	M. LACTILYTICUS*	STREPTOCOCCI AND FACULTATIVELY ANAEROBIC MICROCOCCI†
A	7	7.5
B	60	55
C	37	63
D	10	57
E	13	65
F	90	200
G	17	60
H	55	125
I	7	10
J	24	60
K	340	385
L	24	60
M	53	90
N	6	30
O	3	7
P	60	140
Q	90	135

*Determined from the large colony count on Medium 1.
†Determined from the total colony count on Medium 2.

Medium 2, when inoculated with high dilutions of saliva and incubated anaerobically, yields practically nothing but streptococci and facultatively anaerobic micrococci. *M. lactilyticus* occurs only rarely on plates of this medium.

TABLE II

CHANGES IN pH FOLLOWING THE GROWTH OF A CULTURE OF M. LACTILYTICUS IN 1.0 PER CENT YEAST EXTRACT, 0.5 PER CENT SODIUM LACTATE BROTH AT DIFFERENT INITIAL pH VALUES

INITIAL pH	pH AFTER 24 HOURS' INCUBATION
4.7	no growth
4.8	5.32
5.0	6.0
5.35	6.46
6.20	6.80
7.0	6.92

RESULTS

The results obtained by applying the plating methods described above to saliva collected from seventeen individuals are summarized in Table I. It can be seen that *M. lactilyticus* was present in all of the saliva samples, in numbers varying from three million to 340 million per milliliter. In some cases 50 per cent of the total flora capable of developing under these conditions consisted of this microorganism. These facts leave little doubt that *M. lactilyticus* constitutes a significant portion of the bacterial flora indigenous to human saliva.

Very little is known concerning the ecological relationships of the various groups of microorganisms normally resident in the mouth, but it seems probable that the occurrence of *M. lactilyticus* in this habitat is dependent upon the presence of other bacteria that convert sugars to lactic acid. This appears to be true because *M. lactilyticus* is capable of fermenting only lactate, or the related compounds, pyruvate and malate. Of these substances only lactate would be expected to be present in significant amounts in saliva, and its origin there is undoubtedly due to bacterial fermentation of sugars (Neuwirth and Klosterman, 1940). Since it has been established that *M. lactilyticus* ferments lactate to propionate, acetate, carbon dioxide, and hydrogen (Foubert and Douglas, 1948), it is reasonable to conclude that *M. lactilyticus* carries out a similar biochemical conversion in the mouth. Probably the most significant effect of this fermentation is a resultant increase in pH which is quite marked when the process takes place in an initially acid environment. This is clearly shown in Table II and is due to the fact that the salt of a stronger acid (lactic) is converted to salts of weaker acids (propionic and acetic).

Whether the biochemical activity of *M. lactilyticus* in the mouth is a significant factor in deterring the production of dental caries is not known, but this question deserves the attention of those interested in this particular problem.

The author wishes to express his appreciation to Mr. Richard Layton for his very capable assistance.

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THE UTILIZATION OF CEREALS IN VARIOUS STAGES OF REFINEMENT BY AN ORAL STRAIN OF LACTOBACILLUS ACIDOPHILUS

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THE LITERATURE contains many reports that groups of isolated "primitive" peoples are relatively free from dental caries despite a high content of crude or natural carbohydrates in their diet (Jones, 1935; Osborn and Noriskin, 1937; Waugh and Waugh, 1939). As a result, it has been assumed, by some, that the processing of foods destroys or renders inactive a protective factor against dental caries. In support of this hypothesis, Belding and Belding (1941) maintained that refining makes cereals less suitable for the growth of alkali-producing bacteria which limit the development of acidogenic microorganisms in the oral cavity; Lennox (1941) and Osborn, Noriskin, and Staz (1937) attributed the protective factor to the presence of calcium and phosphorus, both of which are lost to a large extent during the processing of sugar and cereals; Fosdick, Fancher, and Calandra (1942) suggested that the presence of vitamin K in crude sugar cane may act as a deterrent to the caries process.

The ability of the oral acidogenic microorganisms to utilize various forms of refined and unrefined carbohydrates has been the subject of many investigations. In a series of in vitro experiments, Osborn, Noriskin, and Staz (1937) demonstrated that refined cane sugar and refined wheat and corn flour were fermented much more rapidly by acid-producing organisms than the crude forms of the same foods. In contrast, Volker and Pinkerton (1947) found no essential difference in the quantities of acid formed in starch-saliva and in glucose-saliva mixtures when unstimulated saliva from caries-active patients was incubated with various carbohydrate solutions at 37° C. Stephan (1948) reported that the ability of selected oral acidogenic microorganisms to utilize soluble starch in a chemically defined artificial medium is dependent upon the type of carbohydrate and upon the presence of a salivary or bacterial amylase. Clinically, however, there does not appear to be any significant difference between the salivary amylase activity of "caries-active" and "caries-immune" persons (Hess and Smith, 1948).

A consideration of the inability of oral acidogenic microorganisms to utilize various forms and types of carbohydrate must include also the possibility that certain factors conducive to bacterial growth may be either entirely absent, present in insufficient quantities, or present in an unavailable form. This hypothesis was brought into sharp focus by the recent report of Schour and Mass-

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ler (1947). They found that the prevalence of dental caries in Italy after World War II was approximately ½ to ⅐ that in the United States. The diets of the Italian subjects were said to be predominantly carbohydrate in character, "bread" and "pasta" forming the major portion of the food intake. In addition to the low caries incidence, clinical evidence of vitamin B complex deficiency was observed in the majority of the persons examined. These observations conform with the findings that several members of the B group of vitamins, particularly nicotinic acid and thiamin, are essential for carbohydrate metabolism as well as for the maximum growth and acid production of the *Lactobacillus acidophilus* (Spies and Butt, 1942; Weisberger and Johnson, 1946; Dreizen, Scholz, and Spies, 1948). They are also in accord with reports from a number of sources that persons with chronic pellagra and associated deficiencies of the B group of vitamins have a low incidence of dental caries (Sandwith, 1905; Corkill, 1934; Mann, Dreizen, Spies, and Hunt, 1947).

The commonly used cereal grains, corn, wheat, rye, oats, barley, and rice, each contain small quantities of the B group of vitamins and of starch-splitting enzymes. These factors are not distributed evenly throughout the grain. The vitamins tend to be concentrated in the germ and pericarp, the portions usually removed during the refining process (Winton and Winton, 1932; Aykroyd and Swaminathan, 1940; Rosenberg, 1945). In addition, the vitamin and enzyme content of the grain changes as the grain ripens. With these facts in mind and prompted by a desire to study further the relationship between certain dietary elements and dental caries, this investigation was undertaken to determine whether additional sources of certain members of the B group of vitamins and/or amylase are required by an oral strain of *Lactobacillus acidophilus* for the maximum utilization of cereal fractions in various stages of refinement in a chemically defined synthetic medium.

MATERIALS AND METHODS

At the request of the authors, the Research Laboratories of the Quaker Oats Company supplied seventeen cereal products which were used in this study. They consisted of wheat bran, wheat shorts, wheat flour, oat bran, oat shorts, oat middlings, Aveeno,* oat flour, pearled barley, barley second pearl, corn hulls, corn germ, defatted corn germ, degerminated corn meal, rice flour, rye flour, and buckwheat flour. The sample of degerminated corn meal was a white corn product, whereas the other corn derivatives were mixtures of white and yellow corn. Each fraction was supplied in a fine state of subdivision, having been ground on a Raymond pulverizer.

The medium described by Landy and Dicken (1942), was modified for the purpose of this study by excluding glucose from all but the control tubes (Table I). The medium for Assay I was prepared by boiling and refluxing 1.0 Gm. by dry weight of each cereal product in 33 c.c. of distilled water for 5 minutes in a sterile flask. Seventeen cubic centimeters of freshly prepared glucose-free

*Aveeno is made by the air separation of the lighter particles of oat flour from the heavier particles. These lighter particles are characterized by a high protein content and considerable gumlike or gelatin-like characteristics. U. S. Patent No. 2,355,028.

Landy and Dicken medium were added to each flask. Ten cubic centimeters of the resultant mixture were added to each of four tubes. An additional set of four tubes containing glucose instead of a cereal product served as the control. The tubes were autoclaved at 15 pounds' pressure for 15 minutes. Immediately after autoclaving, the tubes were cooled. Two milligrams of Colase D 100, a refined saccharogenic enzyme of 9000 degree Lintner Diastatic Power, were added to each tube and all but the control tubes were then inoculated with the test organism (*Lactobacillus acidophilus*, Hadley, ATCC 4646*).

To determine the effect of the presence or absence of the various members of the B group of vitamins contained in the Landy and Dicken medium (nicotinic acid, thiamin, folic acid, biotin, pantothenic acid, riboflavin, and pyridoxine) and/or amylase, the test medium prepared in the manner described above for Assay I was modified as follows for Assays II through VII:

Assay II: B complex vitamins added; amylase withheld.
Assay III: B complex vitamins withheld; amylase withheld.
Assay IV: B complex vitamins withheld; amylase added.
Assay V: Nicotinic acid and thiamin withheld; amylase added.
Assay VI: Nicotinic acid withheld; amylase added.
Assay VII: Thiamin withheld; amylase added.

TABLE I

COMPOSITION OF TEST MEDIUM*

Casein hydrolysate	2.5 ml.
Salt solution A†	0.5 ml.
Salt solution B‡	0.5 ml.
Sodium acetate	600.0 mg.
Asparagine	25.0 mg.
Tryptophane	10.0 mg.
Cystine	10.0 mg.
Guanine hydrochloride	500.0 μg.
Adenine sulfate	500.0 μg.
Xanthine	500.0 μg.
Uracil	500.0 μg.
Pyridoxine hydrochloride	40.0 μg.
Riboflavin	20.0 μg.
Calcium pantothenate	20.0 μg.
Nicotinic acid	20.0 μg.
Thiamine hydrochloride	10.0 μg.
Folic acid	1.0 μg.
Biotin	0.5 μg.
33 c.c. distilled water containing 1 Gm. carbohydrate	

*Double strength, modified Landy and Dicken medium adjusted to pH 6.8 with NaOH.

†Salt solution A:

K_2HPO_4	5	Gm.
KH_2PO_4	5	Gm.
Water to	50	ml.

‡Salt solution B:

$MgSO_4.7\ H_2O$	10	Gm.
NaCl	0.5	Gm.
$FeSO_4.7\ H_2O$	0.5	Gm.
$MnSO_4.2\ H_2O$	0.337	Gm.
Distilled water to	250	ml.

The *L. acidophilus* inoculum was prepared by transferring from a stab culture in yeast extract dextrose agar to 10 c.c. yeast extract dextrose broth. The inoculated broth was incubated at 37° C. for 24 hours. It was then centri-

*The *Lactobacillus acidophilus* Hadley, strain C_2 (ATCC 4646) has recently been reclassified as *Lactobacillus casei* on the basis of taxonomic studies by Dr. R. P. Tittsler of the Division of Dairy Research, U. S. Department of Agriculture. This is in accord with accumulating evidence that the organism originally designated as the oral *Lactobacillus acidophilus* may be similar to, if not identical with, the *Lactobacillus casei*.

TABLE II

EFFECT OF THE ADDITION OR WITHDRAWAL OF AMYLASE AND/OR B COMPLEX VITAMINS ON ACID PRODUCTION BY LACTOBACILLUS ACIDOPHILUS (HADLEY) IN TEST MEDIUM CONTAINING VARIOUS CARBOHYDRATES

CARBOHYDRATE	ACID PRODUCTION, 72-HOUR READING (C.C. 0.1N NaOH)						
	ASSAY I: B COMPLEX (+) AMYLASE (+)	ASSAY II: B COMPLEX (+) AMYLASE (-)	ASSAY III: B COMPLEX (-) AMYLASE (-)	ASSAY IV: B COMPLEX (-) AMYLASE (+)	ASSAY V: NIACIN (-) THIAMIN (-) AMYLASE (+)	ASSAY VI: NIACIN (-) AMYLASE (+)	ASSAY VII: THIAMIN (-) AMYLASE (+)
1. Glucose	16.50	16.40	0.00	0.00	0.00	0.00	5.10
2. Wheat bran	4.40	0.00	0.00	1.30	1.45	1.40	1.20
3. Wheat shorts	6.30	0.00	0.00	0.20	0.20	0.40	0.40
4. Wheat flour	6.50	0.00	0.00	0.00	0.00	0.00	0.00
5. Oat bran	1.50	0.00	0.00	0.00	0.00	0.00	0.00
6. Oat shorts	1.50	0.00	0.00	0.00	0.00	0.00	0.00
7. Oat middlings	3.60	0.00	0.00	0.00	0.00	0.00	0.00
8. Oat flour	3.80	0.00	0.00	0.00	0.00	0.00	0.00
9. Aveeno	4.40	0.00	0.00	0.00	0.00	0.00	0.00
10. Pearled barley	3.80	0.00	0.00	0.00	0.00	0.00	0.00
11. Barley second pearl	5.00	0.00	0.00	0.00	0.00	0.00	0.00
12. Corn hulls	4.80	0.00	0.00	0.30	0.45	0.30	0.40
13. Corn germ	4.80	0.00	0.00	1.30	1.50	1.30	0.50
14. Corn germ (defatted)	4.90	0.00	0.00	1.40	1.50	1.25	1.40
15. Corn meal (degerminated)	7.00	0.00	0.00	0.00	0.00	0.00	0.00
16. Rice flour	4.90	0.00	0.00	0.00	0.00	0.00	0.00
17. Rye flour	4.80	0.00	0.00	0.00	0.00	0.00	0.00
18. Buckwheat flour	6.50	0.00	0.00	0.00	0.00	0.00	0.00

fuged, the centrifugate decanted, and the cells washed in 10 c.c. of sterile isotonic saline. The washing procedure was repeated four times. The washed cells were resuspended in 10 c.c. sterile saline and shaken. One drop was then transferred to a tube containing 10 c.c. sterile saline. This suspension served as the inoculum.

Each series of tubes in each assay was set up in quadruplicate. One drop of inoculum was added to three tubes in each set, the uninoculated tube serving as a control. The tubes were shaken and incubated at 37° C. Acid production was determined by titration with 0.1N NaOH to the pH of the uninoculated control tube. Readings were taken at the end of 24, 48, and 72 hours. A Beckman pH meter was used for this purpose.

OBSERVATIONS

Assay I.—B complex vitamins added; amylase added. Varying amounts of acid were produced by the test organism from each of the cereal products and from glucose in the presence of the B complex vitamins and synthetic amylase at the end of 72 hours. The greatest amount of acid was produced in the tube containing glucose, the next greatest amount in the tube containing degerminated corn meal, followed by those containing wheat flour, buckwheat flour, wheat shorts, barley second pearl, defatted corn germ, rice flour, corn germ, corn hulls, rye flour, wheat bran, Aveeno, pearled barley, oat flour, oat middlings, oat shorts, and oat bran (Table II). The amount of acid produced in the tube containing degerminated corn meal was equivalent to 42 per cent of that formed in the tube containing glucose.

Assay II.—B complex vitamins added; amylase withheld. The test organism was unable to utilize any of the cereal fractions in the absence of the amylase, although all of the required B complex vitamins were present in the medium. Maximum growth and acid production were observed in the tube containing glucose (Table II).

Assay III.—B complex vitamins withheld; amylase withheld. The growth of the test organism was completely inhibited in each tube in the absence of the B complex vitamins and amylase (Table II).

Assay IV.—B complex vitamins withheld; amylase added. Slight amounts of acid were produced in the tubes containing wheat bran, wheat shorts, corn hulls, corn germ, and defatted corn germ by the end of the experimental period. The greatest amount of acid was detected in the tube containing defatted corn germ. This amount was equivalent to 9 per cent of that produced from glucose in the presence of the essential B complex vitamins in Assay I (Table II).

Assay V.—Nicotinic acid and thiamin withheld; amylase added. Negligible amounts of acid were produced in those tubes containing wheat bran, wheat shorts, corn hulls, corn germ, and defatted corn germ when nicotinic acid and thiamin were withheld from the synthetic medium containing amylase. The cereal fractions fermented to acids in this assay were the same as those involved in Assay IV when all of the essential B complex vitamins were withheld. The

greatest amount of acid was produced from corn germ and defatted corn germ. This amount was equivalent to 9 per cent of that produced in the tube containing the B complex vitamins and glucose in Assay I (Table II).

Assay VI.—Nicotinic acid withheld; amylase added. The results of this assay were similar to those described for Assay V. The same five cereal products were degraded to a slight extent by the acidogenic test organism. The greatest amount of acid was produced in the tube containing wheat bran, an amount equivalent to 9 per cent of that found in the tube containing glucose and the B complex vitamins in Assay I (Table II).

Assay VII.—Thiamin withheld; amylase added. The absence of thiamin exerted an effect similar to that observed in Assays V and VI, wheat bran, wheat shorts, corn hulls, corn germ, and defatted corn germ being utilized to a slight degree by the test organism. The greatest amount of acid produced from a test cereal was encountered in the tube containing defatted corn germ. It was equivalent to 9 per cent of that produced in the tube containing glucose and the essential B complex vitamins in Assay I. In addition, submaximal growth was observed in the tube containing glucose, acid production in this tube being equivalent to 35 per cent of the amount formed when thiamin was included in the test medium as in Assay I (Table II).

DISCUSSION

The findings demonstrate that under the conditions of this study external sources of amylase and of certain members of the B group of vitamins are required for the maximum utilization of various cereal fractions by an oral strain of *Lactobacillus acidophilus* in a synthetic medium containing all of the other known growth requirements of this organism. None of the seventeen cereal products in this study were utilized by the test organism in the absence of an external source of amylase. Naturally occurring amylase has been recovered from oats, barley, wheat, corn, rice, and rye during germination. In these cereals, the amylase content is reduced considerably during the ripening process (Winton and Winton, 1932; Burkholder, 1943). In addition, nearly all enzymes are irreversibly destroyed by heating to 80° C. (Sumner and Somers, 1947). The amount, if any, of amylolytic enzyme still retained in different parts of the grain after boiling and autoclaving was not sufficient to promote the degradation of the cereal fractions used in this study into more simple carbohydrates. The findings reveal also that under the conditions employed, the test organism does not secrete or contain an amylase in amounts sufficient to convert starch into utilizable sugar.

The cereal products in the amounts used did not, for the most part, contain members of the B group of vitamins, particularly nicotinic acid and thiamin, in the quantities or in the form required for the maximum growth and acid production of the test organism. The significance of these vitamins in the utilization of cereals was emphasized by the observation that slight amounts of acid were produced from several fractions which are naturally rich in these vitamins, although synthetic vitamins were withheld from the test medium.

These fractions comprised wheat bran, wheat shorts, corn germ, defatted corn germ, and corn hulls. Wheat shorts, in addition to containing bran fragments, also contain the bulk of the wheat germ. It will be noted that each of these products is derived from either the cereal germ or pericarp, the portions usually removed during the refining process. The quantities, however, were inadequate to promote the maximum growth and acid production of the test organism in the absence of an external source of required members of the B group of vitamins.

The utilization of cereals by the test organism was dependent to a large extent upon the quantity and the type of starch contained in the cereal. It was observed that, in general, the more refined the cereal, the greater the amount of acid produced, provided that amylase and the required B complex vitamins were present in the medium. The values obtained for Aveeno and for the pearlings from the second barley pearler were not in accord with this trend. Since the materials in question were not derived from the same original sample of grain, it may be that there is a wide range of values between different samples of the same cereal. The samples of refined corn, wheat, buckwheat, barley, rice, rye, and oats were fermented in a decreasing order. Oat bran and oat shorts were acted upon but slightly by the test organism, suggesting the possibility that a factor antagonistic to the growth of the oral *L. acidophilus* may be present in these fractions.

SUMMARY AND CONCLUSIONS

A study of the utilization of seventeen different cereal fractions and glucose by an oral strain of the *L. acidophilus* in a chemically defined synthetic medium shows the following:

1. An external source of some form of amylase is required for the maximal utilization of cereals by the oral *L. acidophilus* (Hadley). Under the conditions of this study, neither the cereal fractions nor the test organism, in the amounts used, contained amylase in amounts sufficient to promote the breakdown of polysaccharides.

2. An external source of nicotinic acid and thiamin is required for the maximum utilization of cereals by the oral *L. acidophilus* (Hadley). Several fractions derived from corn and wheat, comprising the germ or pericarp of the grain, were utilized to a slight extent by the test organism when nicotinic acid and thiamin were withheld from the synthetic medium. Presumably this indicates that the germ and pericarp of these grains, the portion richest in vitamin B content, contain nicotinic acid and thiamin in amounts sufficient to promote minimal growth of this organism.

3. In the presence of an amylase and of adequate amounts of essential members of the B group of vitamins, the degree of acid production was, for the most part, directly related to the degree of refinement of the cereal grain.

4. These findings suggest that in the absence of nicotinic acid and thiamin or substances that act similarly in human saliva, the ingestion of large amounts of refined carbohydrates may not be in and of themselves necessarily conducive to dental caries.

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FLUORINE, ASH, CALCIUM, AND PHOSPHORUS IN HUMAN TEETH

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ALTHOUGH the actual relationship remains unknown, it is evident from previous publications,[1-5] and it is apparent in the data of this report, that fluorine accumulates in human teeth when fluoride drinking waters are being ingested. When they develop in such an environment, human teeth may contain as much as 0.05 to 0.20 per cent fluorine in the dentin and 0.03 to 0.10 per cent in the enamel. These quantities of fluorine are characteristic, therefore, of severely and perhaps moderately mottled teeth. In general, human enamel formed under a nonfluoride environment will average approximately 0.010 per cent fluorine and dentin will average 0.023 per cent fluorine.[6, 7]

Aside from fluorine, mottled (fluorosed) human teeth have not been studied very extensively for other chemical changes. Bowes and Murray[2] found no significant differences in the calcium, phosphorus, and magnesium content of mottled vs. nonmottled teeth. Other results, including iron, are likewise negative.[8] Spectrographic analyses of human dentin and enamel reported by Lowater and Murray[3] indicated that "potassium was not found generally but was present in enamel and dentin of 'mottled teeth' and in fluoride-fed rats' teeth." The iron content was also diminished in the enamel of fluoride-fed rats. Fluorine varying from 0.029 per cent to 0.095 per cent in swine rations increased the total tooth fluorine up to 0.375 per cent but chemical analyses revealed no concomitant effect on ash, calcium, phosphorus, magnesium, or carbon dioxide content.[9] Rats' molar teeth also did not vary in ash, calcium, and phosphorus, although the tooth fluorine varied from 0.0310 per cent up to 0.1842 per cent.[10] However, the incisor teeth of rats given rations containing 0.15 per cent sodium fluoride in the diet decreased in total ash, even on a relatively high calcium intake.[11] Fluorine in similarly excessive quantities (0.1 per cent sodium fluoride in the ration) reduced ash and phosphorus, and increased slightly the calcium in rats' incisor teeth.[12] Fluorine acquired by the enamel and dentin of dogs' teeth after tooth eruption, i.e., "secondary fluorine,"[13] was not associated with any change of ash in the enamel or dentin.[14]

The bones of animals and human beings generally are quite susceptible to an alteration in calcification as a result of fluoride ingestion.[9, 12, 15, 16] Thus, 0.03 per cent and 0.06 per cent sodium fluoride in rats' diets increased the ash content of bones and decreased the calcium content of the ash.[15] The level of calcium in rats' rations appears to be a predetermining factor in the effect of fluorine on bone ash.[11] The classical studies by Roholm[16] established the effect of excessive fluorine on human bone calcification.

In the previous studies of the ash, calcium, and phosphorus in mottled vs normal human teeth the variations in fluorine content have not been very pronounced.[2, 3] Thus, the pooled mottled teeth analyzed by Bowes and Murray[2]

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and the spectrographic analyses of Lowater and Murray[3] were made on normal (London, England) enamel and dentin containing 0.025 per cent fluorine vs. mottled enamel and dentin (from Maldon, England) containing 0.032 per cent and 0.073 per cent fluorine, respectively. The spectrographic studies by Boissevain and Drea[1] were made on normal enamel (0.0 per cent fluorine) vs. mottled enamel (0.063 per cent fluorine) and on normal dentin (0.068 per cent fluorine) vs. fluorosed dentin (0.112 per cent fluorine).

It was desirable, therefore, to accumulate additional data relative to the relation of dental fluorosis to calcification of dental tissues, which would represent wider variations of fluorine in the teeth and which would be related to the specific fluorine content of the teeth rather than to the mottled enamel diagnosis. It was sought to ascertain also the relation of a moderate or nonpathologic quantity of fluorine in dentin and enamel to the ash, calcium, and phosphorus content.

The human teeth analyzed in this study were extracted from individuals living in several fluoride and nonfluoride areas, all the highly fluorosed teeth, beyond reasonable doubt, having acquired fluorine by prolonged ingestion of fluoride drinking waters. Both sound and carious teeth were included, previous data,[17, 18, 19] as well as our own data (Table I), having shown no relation of calcium, phosphorus, and ash to caries experience. Similarly the age of the teeth was ignored since most previous data[18, 19] have shown (as do our own data in Table I) no relation of age to ash, calcium, and phosphorus. There was no possibility of differentiating between fluorine acquired before vs after the eruption of these teeth, although admittedly these two types of acquired dental fluorine, i.e., "primary" and "secondary,"[13] might react differently.

TABLE I

ASH, CALCIUM, AND PHOSPHORUS CONTENT OF DENTIN AND ENAMEL RELATED TO CARIES

	NO. OF TEETH	AGE (YR.)	ASH (%)	CA (%)	P (%)	CA:P (RATIO)
Enamel of sound teeth	30	45 ± 2.33*	95.1 ± .164	36.7 ± 251	17.5 ± .071	2.10 ± .019
Enamel of carious teeth	45	42 ± 2.15	94.6 ± .106	37.1 ± .116	17.4 ± .036	2.13 ± .069
Dentin of sound teeth	28	45 ± 2.33	77.2 ± .403	29.3 ± .182	14.0 ± .074	2.09 ± .011
Dentin of carious teeth	45	42 ± 2.15	76.3 ± .370	29.2 ± .180	14.0 ± .069	2.09 ± .008
Enamel aged 16.40 yr.	24	27 ± 1.32	94.6 ± .136	36.9 ± .169	17.4 ± .056	2.12 ± .010
Enamel aged 40-60 yr.	44	53 ± 0.84	94.6 ± .104	37.4 ± .101	17.4 ± .028	2.15 ± .006
Dentin aged 16-40 yr.	24	27 ± 1.32	76.5 ± .506	29.1 ± .199	14.1 ± .099	2.07 ± .011
Dentin aged 40-60 yr.	44	53 ± 0.84	76.3 ± .316	29.4 ± .184	14.0 ± .059	2.10 ± .008

*$\sigma_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

The teeth were cleaned, all carious material removed, the crowns separated, dried, broken into small pieces, extracted with alcohol and ether, and pulverized to pass a 60-mesh sieve. The dentin and enamel were separated according to the Manly-Hodge technic[20] and ash, calcium, and phosphorus were determined by standard analytical methods.[21, 22]

The data presented in Table I conform with previous data[16, 17, 18] and indicate, as mentioned above, no differences in ash, calcium, and phosphorus in carious vs. noncarious teeth and no relation of ash, calcium, and phosphorus to tooth age.

In Table II are the means of the analytical results arbitrarily grouped according to the fluorine content of the dentin and enamel. As it appears from these data, there is no change in enamel ash, calcium, phosphorus, and the calcium-phosphorus ratio, although the mean enamel-fluorine varied from 0.006 per cent to 0.049 per cent. Although the mean fluorine in dentin varied from a low value of 0.012 per cent up to 0.125 per cent there was no change in the total calcium and phosphorus or the calcium-phosphorus ratio. There is the suggestion (Table II), however, that the ash content of the highly fluorosed dentin is above normal. Comparing the mean ash figure, i.e., 80.1 ± 0.668 per cent ash in dentin (which averaged 0.125 ± .0097 per cent fluorine) with 77.6 ± 0.345 per cent ash in dentin (which contained 0.021 ± 0.0007 per cent fluorine), the difference is 2.5 ± 0.751 per cent which is statistically significant.

TABLE II

ASH, CALCIUM, AND PHOSPHORUS IN DENTIN AND ENAMEL IN RELATION TO FLUORINE CONTENT

NUMBER OF TEETH ANALYZED	RESULTS OF ANALYSIS OF DENTIN AND ENAMEL				
	F (%)	ASH (%)	CA (%)	P (%)	CA:P (RATIO)
	Fluorine in dentin = 0.007 – 0.014 per cent				
15	0.012 ± 0.0006*	77.4 ± 0.592	29.5 ± 0.225	14.1 ± 0.096	2.10 ± 0.016
	Fluorine in dentin = 0.015 – 0.030 per cent				
39	0.021 ± 0.0007	77.6 ± 0.345	29.5 ± 0.141	14.0 ± 0.073	2.11 ± 0.011
	Fluorine in dentin = 0.031 – 0.050 per cent				
9	0.038 ± 0.0020	76.9 ± 0.850	29.5 ± 0.336	13.9 ± 0.088	2.12 ± 0.017
	Fluorine in dentin = 0.076 – 0.213 per cent				
20	0.125 ± 0.0097	80.1 ± 0.668	29.1 ± 0.188	14.6 ± 0.191	2.01 ± 0.031
	Fluorine in enamel = 0.003 – 0.009 per cent				
22	0.006 ± 0.0004	95.4 ± 0.186	36.5 ± 0.281	17.5 ± 0.082	2.08 ± 0.020
	Fluorine in enamel = 0.010 – 0.025 per cent				
22	0.015 ± 0.0010	95.8 ± 0.131	37.1 ± 0.297	17.6 ± 0.095	2.11 ± 0.026
	Fluorine in enamel = 0.030 – 0.100 per cent				
19	0.049 ± 0.004	95.9 ± 0.233	36.3 ± 0.349	17.8 ± 0.129	2.05 ± 0.025

*$\sigma_M = \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

While a slight increase of the ash in dentin therefore may be a characteristic of moderate and severely mottled human teeth (0.030 to 0.100 per cent or more fluorine), there is no evidence that lesser quantities of fluorine in the dentin will affect the ash or calcium and phosphorus content. Slight increases in enamel fluorine and dentin fluorine, however, are expected (although not demonstrated) to occur where drinking waters contain upwards of 1.0 to 1.5 ppm fluorine. Under such fluoride drinking water exposures, the dental caries experience is reduced some 60 to 65 per cent in the 12- to 14-year-old population.[23] There is no explanation as to the mechanism of this anticaries effect

of fluoride drinking waters. It would appear, however, from the data in Table II that slight accumulations of fluorine in those teeth which are noncarious because of a fluoride water exposure* do not alter the ash, calcium, or phosphorus content. These data furnish no indication, therefore, that the explanation of the fluorine anticaries effect is related to the total quantities of ash, calcium, and phosphorus or the calcium-phosphorus ratio of the enamel and dentin.

As has been pointed out previously[2] mottled enamel "is not due to diminished calcification." The data accumulated in this current study agree with this previous observation and also extend this conclusion to include teeth which may contain slightly increased quantities of fluorine but are nevertheless not mottled. Speculation continues none the less on the probable effect of fluorine ingestion on the type and physical properties of the apatite complex laid down in enamel and dentin during tooth calcification.

SUMMARY

1. The ash, calcium, and phosphorus content of human enamel and dentin did not vary with caries experience or age.

2. An eightfold increase in enamel fluorine was not related to any change in enamel ash, calcium, or phosphorus.

3. A tenfold increase in dentin fluorine was not related to any change in dentin, calcium, and phosphorus. There was a slight increase in the ash content of highly fluorosed dentin.

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*Referring to Table II it may be suggested that 0.015 – 0.030 per cent dentin fluorine and 0.010–0.025 per cent enamel fluorine eventually may prove to be a characteristic feature of such noncarious teeth.

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THE FAILURE OF AMMONIUM IONS TO INHIBIT THE GROWTH OF ORAL LACTOBACILLI

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EVIDENCE in support of the etiological significance of lactobacilli in dental caries is based mainly on indirect observations, such as the repeatedly corroborated positive correlation between high lactobacillus counts and caries activity.[1-5] Definition of factors which either allow or prohibit propagation of lactobacilli in the oral cavities of various individuals would be highly important from a practical as well as a theoretical standpoint.

In the past, several groups of workers have attempted to obtain evidence concerning the presence in saliva of factors acting as inhibitors of lactobacilli.[6-10] The most recent attempts along these lines are those of Kesel, O'Donnell, Kirch, and Wach[11, 12] who have claimed that ammonium ions in low concentrations exert a toxic effect on oral lactobacilli. These workers believe that the presence of ammonium ions in the saliva of certain individuals is an important factor in inhibiting the growth of lactobacilli and consequently in reducing caries activity. This conclusion was arrived at mainly as the result of the following observations: (1) saliva from caries-immune persons contained a lactobacillus-inhibiting substance which was tentatively identified as ammonia, and (2) the addition of small amounts of ammonium carbonate or dibasic ammonium phosphate to cultures of the Hadley strain of *Lactobacillus acidophilus* exerted a marked bactericidal effect.

To anyone familiar with the facts of bacterial nutrition it is surprising that the small concentration of ammonium ions employed by Kesel, O'Donnell, Kirch, and Wach[11, 12] should exert a toxic effect on lactobacilli. In view of the active interest in dentifrices and chewing gum containing ammonium ions which has arisen directly from the work of Kesel and his collaborators, it was considered desirable to reinvestigate the effect of ammonium ions on lactobacilli isolated from the oral cavity.

METHODS

Culture media.—Medium 1 consisted of Bacto-tryptone, 20 Gm.; Bacto-yeast extract, 10 Gm.; glucose, 10 Gm.; filtered tomato juice rendered free of sugar by yeast fermentation, 100 ml.; and tap water, 900 ml. The pH of this medium was 6.8. Medium 2 consisted of beef infusion broth containing 1 per cent glucose and 1 per cent Bacto-peptone. The pH was adjusted to 7.0. Hadley's modification of Kulps' tomato agar[1] was used in isolating and purifying the lactobacilli tested.

Identity of cultures.—Instead of following the dubious practice of labeling all cultures of lactobacilli obtained from the oral cavity as "*L. acidophilus*" an attempt was made to identify our cultures as closely as possible. Optimum

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growth temperatures were determined by incubating cultures in Medium 1 at 25° C., 30° C., 37° C., and 45° C. for 5 days and measuring the growth response either by titration of the acid formed or turbidimetrically using the Klett-Summerson photoelectric colorimeter. Both methods gave comparable results. The fermentation of different carbohydrates was determined by substituting the various sugars at 1 per cent concentration for the glucose in Medium 1 and titrating the acid formed after 5 days' incubation at 37° C. Optical activity of the lactic acid formed was determined by polarizing ether extracts of 7-day-old cultures grown in Medium 1. Cultures grown in Medium 1 in Eldredge tubes served to demonstrate the heterofermentative or homofermentative nature of the organisms.

Effect of ammonium ions on growth.—This was determined in both Mediums 1 and 2 as follows. The solutions of ammonium carbonate and dibasic ammonium phosphate used were adjusted to pH 7.5 and sterilized by nitrogen pressure filtration through sintered glass filters before addition to the medium. One-tenth to 1.0 ml. amounts of the ammonium carbonate or ammonium phosphate solutions of appropriate concentration were added aseptically to 9.0 ml. of sterile medium. The volume of liquid in each tube was then adjusted to 10.0 ml. where necessary with sterile tap water, and 0.1 ml. of a 24-hour culture of the test organism grown in Medium 1 was added. Following incubation at 37° C. for 24 to 40 hours the growth response was measured with the photoelectric colorimeter.

The amount of inoculum used had no effect upon the outcome of the experiments. Identical results were obtained whether the inoculum consisted of 0.1 ml. or a loopfull per tube.

CULTURES EMPLOYED

Cultures 1, 2, and 3 were isolated from material removed from a carious lesion of one individual. Cultures 4, 5, 7, and 8 were isolated from the saliva of four individuals.

Culture 6 is the Hadley strain of *L. acidophilus,* and was obtained from the American Type Culture Collection where it is carried as number 4646. When received by us it was labeled "*L. casei,* received as *L. acidophilus.*"

All of the cultures produce smooth, compact colonies with the exception of Culture 8 which is slightly rough. Morphologically, the cultures are all gram-positive, straight rods of variable length. Strain 8 occasionally produces slightly curved cells. All of the cultures are catalase negative and facultatively anaerobic.

Table I lists some of the physiological characteristics of the above organisms. Cultures 1, 2, and 3 appear to be similar to *L. acidophilus* with the important exceptions that litmus milk is not coagulated and the colonial form is smooth rather than rough as described for this species. Culture 4 differs from the above three inasmuch as it ferments maltose and fails to ferment lactose. We are uncertain as to the exact taxonomic position of these four organisms. Culture 5 appears to be identical with *L. lactis,* while Culture 6 has the characteristics of *L. casei.* Cultures 7 and 8 are heterofermentative lactobacilli while

all of the other isolates are homofermentative. Culture 7 has a high optimum growth temperature and is probably *L. fermenti*, while we have classified Culture 8 as *L. buchneri*.

TABLE I
PHYSIOLOGICAL CHARACTERISTICS OF LACTOBACILLI EMPLOYED

CULTURE NUMBER	OPTIMUM TEMPERATURE, ° C.	OPTICAL ACTIVITY OF LACTIC ACID	GLUCOSE	CARBOHYDRATE FERMENTATIONS			
				ARABINOSE	SUCROSE	MALTOSE	LACTOSE
1	37	inactive	+	−	+	−	+
2	37	inactive	+	−	+	−	+
3	37	inactive	+	−	+	−	+
4	37	inactive	+	−	+	+	−
5	37	levorotatory	+	−	+	+	+
6	30 to 37	inactive	+	−	+	+	+
7	45	*	+	−	+	+	+
8	37	*	+	−	+	+	+

*Not determined.

EXPERIMENTS

The effect of ammonium ions on growth was first evaluated using Cultures 5, 6, and 7. Duplicate sets of ten tubes of Medium 1 containing ammonium carbonate in amounts ranging from 0.56 to 5.6 mg. per tube, and control tubes of Medium 1 containing no ammonium carbonate, were inoculated with 0.1 ml. amounts of 24-hour cultures of the respective organisms. After overnight incubation at 37° C., abundant growth had occurred in all of the cultures, and turbidity readings made after 40 hours of incubation showed that the addition of ammonium carbonate had exerted no effect on growth of any of the test organisms. This was in marked contrast to the findings of Kesel, O'Donnell, Kirch, and Wach[11, 12] who reported that the addition of as little as 0.56 mg. of ammonium carbonate to Culture 6 in beef-broth glucose medium exerted a striking inhibition of growth, while 1.12 mg. of ammonium carbonate per tube completely destroyed the organisms.

TABLE II
THE EFFECT OF AMMONIUM CARBONATE ON GROWTH OF CULTURE 6 IN MEDIUM 1

AMMONIUM CARBONATE PER TUBE MG.	COLORIMETER READINGS IN SCALE DIVISIONS
.0	298
5.6	298
11.2	320
16.8	310
22.4	294
28.0	290
33.6	276
39.2	284
44.8	262
49.4	250
56.0	260

In order to determine whether ammonium carbonate in any reasonable concentration at all could inhibit the growth of Culture 6 the above experiment was repeated using concentrations of ammonium carbonate 10 times those

employed in the first experiment. The results of this experiment are summarized in Table II and show that no appreciable inhibition of growth was apparent even in the presence of 56.0 mg. of ammonium carbonate per tube.

As our test medium in the above experiments differed from that employed by Kesel, O'Donnell, Kirch, and Wach[11, 12] it was conceivable that this could account in part for our widely different results. To test this possibility the inhibition tests were repeated using Medium 2 (beef infusion, peptone, glucose broth), as employed by Kesel, O'Donnell, Kirch, and Wach. All of the eight test organisms were employed in this experiment in order that information concerning the effect of ammonium ions on a wide variety of oral lactobacilli might be obtained. A single concentration, 25 mg. per tube of ammonium carbonate, sodium carbonate, or dibasic ammonium phosphate, was employed, together with control tubes of medium containing no added test substance. The results of this experiment which are summarized in Table III again gave no evidence which could be interpreted as indicating inhibition of growth due to ammonium ions. The growth of Cultures 7 and 8 was inhibited to some extent in the presence of dibasic ammonium phosphate, but a similar inhibition of these cultures was produced by sodium carbonate. The growth of the other six cultures in the presence of either of the two ammonium salts used equaled or exceeded the growth in control media containing no added test substance.

TABLE III

EFFECT OF SODIUM CARBONATE, AMMONIUM CARBONATE, AND DIBASIC AMMONIUM PHOSPHATE ON GROWTH OF LACTOBACILLI IN MEDIUM 2

ADDITION	CULTURE NUMBER							
	1	2	3	4	5	6	7	8
	COLORIMETER READINGS IN SCALE DIVISIONS							
None	177	167	155	167	95	143	90	94
25 mg. Sodium carbonate	195	120	142	170	62	80	45	45
25 mg. Ammonium carbonate	226	190	210	212	105	181	97	78
25 mg. Dibasic ammonium phosphate	165	222	176	180	93	188	30	41

DISCUSSION

Our results concerning the effect of ammonium ions on the growth of lactobacilli from the oral cavity are markedly different from those obtained by Kesel, O'Donnell, Kirch, and Wach[11, 12] who found that low concentrations of ammonium carbonate or ammonium phosphate possessed pronounced bactericidal properties toward these organisms. As one might have predicted from a knowledge of bacterial nutrition, our results show that ammonium salts added to culture media in concentrations of 0.25 to 0.5 per cent produce no inhibition of the growth of the strains of lactobacilli tested.

Our in vitro findings obviously do not support the concept that ammonium ions as such, whether normally present in saliva or added artificially, are an important factor in preventing the growth of lactobacilli in the oral cavity, although we have not confirmed this in vivo.

The effectiveness of dentifrices containing ammonium ions as caries prophylactics must of course be determined by extensive clinical trials. Should these

new dentifrices prove to be effective in controlling dental caries, it is of great practical as well as theoretical importance that their mechanism of action be elucidated. It is our opinion that any effectiveness that these materials may subsequently be shown to possess must be explained on some basis other than inhibition of lactobacilli by ammonium ions.

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STUDIES ON THE INCIDENCE AND CAUSE OF DENTAL DEFECTS IN CHILDREN

IX. The Deciduous Molars: Fissures and Caries

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THE relationship of fissures to the causation of caries of the first permanent molars was stressed by a number of investigators.[1-5] Very little is known, however, of the relation of fissures to caries occurrence in the deciduous molars. As a matter of fact, statistical reports on the incidence of fissures on the occlusal surfaces of the primary molars seem to be wanting. Yet the opinion is held that the initiation of the carious process in these teeth is dependent upon the presence or absence of these defects.[6, 7] In theory this may sound plausible, but it is important to learn whether clinical observations bear out such assumptions.

No general agreement exists as to the exact definition of the term "fissure." Of the explanations that are at hand that of a developmental fault usually found on the occlusal surface of a tooth and caused by an imperfect union of the abutting enamel coverings seems to be the most acceptable.

THE FIRST DECIDUOUS MOLARS

In this study of a group of 2,211 white and Negro children of both sexes, whose ages ranged from 4 to 14 years, a total of 2,221 noncarious first deciduous molars were considered (Table I). First deciduous molars were declared noncarious if at the time of examination they failed to show dental decay. Those which had been filled were not included in this category, for their past history could not be definitely ascertained. This investigation therefore concerns itself with an exposition of findings as disclosed by a most careful examination of 2,221 first deciduous molars free from caries on which no dental work had ever been performed. The selection of such teeth for purposes of study was based on the conclusion that in no other way can the incidence of this defect be accurately determined. The explorer, mirror, college pliers, cotton pellets, and scalers were used in the examination. The x-ray was not employed.

Data

Of the total number of 1,087 noncarious first deciduous molars examined in the white boys and girls, 134 disclosed fissures. The Negro children (male and female) showed a lower percentage of such defective teeth. Out of a total of 1,134 first deciduous molars inspected in them, 79 showed inadequate coalescence of the abutting enamel coverings (Table II).

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TABLE I

AGE DISTRIBUTION IN WHITE AND NEGRO CHILDREN WITH NONCARIOUS DECIDUOUS MOLARS WITH FISSURES

AGE AT LAST BIRTHDAY	WHITE BOYS	WHITE GIRLS	NEGRO BOYS	NEGRO GIRLS	ALL GROUPS COMBINED
4	8	5	6	2	21
5	41	36	24	40	141
6	59	54	29	37	179
7	43	47	46	51	187
8	79	61	54	48	242
9	56	53	33	37	179
10	67	47	39	26	179
11	40	26	17	24	107
12	20	22	8	13	63
13	9	3	6	5	23
Total	422	354	262	283	1,321

There was a much higher incidence of fissures on the occlusal surfaces of these teeth in the white girls than in the white boys. However, in the Negro girls and boys the occurrence of fissures was nearly equal. The influence of sex and race on the causation of these faults in the enamel appeared complicated. The reason for these variations and equalities is difficult to conceive and the mode of action must yet be determined.

TABLE II

INCIDENCE OF FISSURES IN NONCARIOUS FIRST DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (ALL AGES COMBINED)*

TOTAL NUMBER OF BOYS EXAMINED: WHITE 681 NEGRO *435*	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	RIGHT	LEFT	TOTAL	
Number of noncarious first deciduous molars examined	192 *158*	207 *160*	399 *318*	140 *108*	127 *114*	267 *222*	666 *540*
Number of noncarious first deciduous molars with fissures	17 *5*	13 *9*	30 *14*	15 *10*	16 *14*	31 *24*	61 *38*
Per cent of noncarious first deciduous molars with fissures	8.9 *3.2*	6.3 *5.6*	7.5 *4.4*	10.7 *9.2*	12.5 *12.3*	11.6 *10.8*	9.1 *7.0*

TOTAL NUMBER OF GIRLS EXAMINED: WHITE 615 NEGRO *480*	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	RIGHT	LEFT	TOTAL	
Number of noncarious first deciduous molars examined	124 *172*	116 *168*	240 *340*	94 *133*	87 *121*	181 *254*	421 *594*
Number of noncarious first deciduous molars with fissures	23 *8*	24 *9*	47 *17*	9 *10*	17 *14*	26 *24*	73 *41*
Per cent of noncarious first deciduous molars with fissures	18.5 *4.7*	20.7 *5.4*	15.4 *5.0*	9.6 *7.5*	19.5 *11.6*	14.4 *9.4*	17.1 *6.9*

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

TABLE III

GENERAL SUMMARY: INCIDENCE OF FISSURES IN NONCARIOUS FIRST DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (ALL AGES COMBINED)

TOTAL NUMBER OF PUPILS EXAMINED (WHITE AND NEGRO BOYS AND GIRLS): 2,211	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	RIGHT	LEFT	TOTAL	
Total number of noncarious first deciduous molars examined	646	651	1297	475	449	924	2221
Total number of noncarious first deciduous molars with fissures	53	55	108	44	61	105	213
Per cent of non-carious first deciduous molars with fissures	7.9	8.4	8.3	9.3	13.6	11.4	9.6

In the white girls the upper left molar was the tooth mostly affected. In the white boys, however, as well as in the Negro boys and girls the lower left molar was more frequently involved. Bilaterality in fissure incidence appeared less constant than in the case of caries of these teeth in both sexes and races. Its variation was especially striking in the lower right and left molars of the white girls. In general the lower molars showed a higher incidence of fissures than their upper antagonists (Table III).

TABLE IV

INCIDENCE OF FISSURES IN NONCARIOUS SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (ALL AGES COMBINED)*

TOTAL NUMBER OF BOYS EXAMINED: WHITE 681 / NEGRO *435*	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	RIGHT	LEFT	TOTAL	
Number of noncarious second deciduous molars examined	172 *142*	177 *142*	349 *284*	130 *115*	129 *110*	259 *225*	608 *509*
Number of noncarious second deciduous molars with fissures	17 *12*	11 *12*	28 *24*	9 *9*	9 *9*	18 *18*	46 *42*
Per cent of noncarious second deciduous molars with fissures	9.9 8.5	6.2 8.5	8.0 *8.5*	6.9 *7.8*	7.0 *8.2*	6.9 *8.0*	7.6 *8.3*

TOTAL NUMBER OF GIRLS EXAMINED: WHITE 615 / NEGRO *480*	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	LEFT	RIGHT	TOTAL	
Number of noncarious second deciduous molars examined	126 *176*	125 *157*	251 *333*	98 *121*	88 *113*	186 *234*	437 *567*
Number of noncarious second deciduous molars with fissures	7 *18*	17 *21*	24 *39*	3 *7*	9 *7*	12 *14*	36 *53*
Per cent of noncarious second deciduous molars with fissures	5.6 *10.2*	13.6 *13.4*	9.6 *11.7*	3.1 *5.9*	10.2 *6.0*	6.4 *6.0*	8.5 *9.3*

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

THE SECOND DECIDUOUS MOLARS

Source of Material and Data

This study was conducted on the same group of 2,211 white and Negro children. It involved the consideration of 2,121 noncarious second deciduous molars. Of this number, 193 showed fissures.

Of the total number of 1,045 noncarious second deciduous molars investigated in the white boys and girls, 82 had fissures. The Negro children (male and female) revealed a slightly higher incidence. Out of a total of 1,075 second deciduous molars inspected in them, 111 disclosed imperfect union of the adjacent dental lobes. In both white and Negro boys the fissure incidence was slightly lower than in the white and Negro girls. Sex and race offer here, too, a theme for special study. The factor responsible for the production of the various differences as well as similarities must yet be resolved (Table IV).

It was observed that in the white boys the upper right second deciduous molar was most often involved. In the white and Negro girls, however, the upper left molar showed the highest incidence of this defect. The fissure prevalence in the Negro boys was almost identical for the upper and lower molars of both sides (right and left). Lack of bilaterality was rather constant in the white boys and girls. Bilaterality, however, was demonstrated in the Negro children (male and female). In general, the upper molars had a higher fissure incidence than their lower antagonists (Table V).

TABLE V

GENERAL SUMMARY: INCIDENCE OF FISSURES IN NONCARIOUS SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (ALL AGES COMBINED)

TOTAL NUMBER OF PUPILS EXAMINED (WHITE AND NEGRO BOYS AND GIRLS): 2,211	UPPER MOLARS			LOWER MOLARS			GRAND TOTAL
	RIGHT	LEFT	TOTAL	RIGHT	LEFT	TOTAL	
Total number of noncarious second deciduous molars examined	616	601	1217	464	440	904	2121
Total number of noncarious second deciduous molars with fissures	54	61	115	28	50	78	193
Per cent of noncarious second deciduous molars with fissures	8.8	10.1	9.4	6.0	11.4	8.6	9.1

DISCUSSION

This study revealed a practically equal fissure incidence in both first and second deciduous molars. (First molars 9.6 per cent; second molars 9.1 per cent.) Claims that the second deciduous molar is more prone to decay because its occlusal surface is more frequently the seat of imperfect coalescence of the enamel lobes were not substantiated. The view that first deciduous molars are lacking in pits and fissures and that this accounts for their lesser susceptibility to caries was not corraborated. The impression may be formed that the greater fissure incidence in the lower first deciduous molars is responsible for

the increased caries prevalence in these teeth. There does not seem to be the slightest basis for this theory. The fissure incidence in the upper second deciduous molars exceeded that in the lower, yet the caries incidence was higher in the latter. This means that there is no relationship between the occurrence of caries and that of fissures in these teeth.

TABLE VI

INCIDENCE OF FISSURES IN NONCARIOUS FIRST AND SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 4 TO 6 INCLUSIVE)

FIRST MOLARS			
AGES AT LAST BIRTHDAY	NUMBER OF PUPILS EXAMINED	NUMBER OF NONCARIOUS FIRST DECIDUOUS MOLARS EXAMINED	NUMBER OF NONCARIOUS FIRST DECIDUOUS MOLARS WITH FISSURES
4	21	73	4
5	141	411	15
6	178	470	35
Total	340	954	54

SECOND MOLARS			
AGES AT LAST BIRTHDAY	NUMBER OF PUPILS EXAMINED	NUMBER OF NONCARIOUS SECOND DECIDUOUS MOLARS EXAMINED	NUMBER OF NONCARIOUS SECOND DECIDUOUS MOLARS WITH FISSURES
4	21	60	7
5	141	369	22
6	178	397	23
Total	340	826	52

TABLE VII

PERSISTENCE OF FISSURES IN NONCARIOUS FIRST AND SECOND DECIDUOUS MOLARS IN WHITE AND NEGRO BOYS AND GIRLS. (AGES 10 TO 13 INCLUSIVE)*

	WHITE AND NEGRO BOYS			WHITE AND NEGRO GIRLS		
AGE AT LAST BIRTHDAY	NUMBER OF CHILDREN EXAMINED	NUMBER OF FIRST DECIDUOUS MOLARS WITH FISSURES	NUMBER OF SECOND DECIDUOUS MOLARS WITH FISSURES	NUMBER OF CHILDREN EXAMINED	NUMBER OF FIRST DECIDUOUS MOLARS WITH FISSURES	NUMBER OF SECOND DECIDUOUS MOLARS WITH FISSURES
10	67	11	8	47	2	3
	39	*3*	*10*	*26*	*2*	*6*
11	40	4	1	26	1	4
	17	*0*	*4*	*24*	*1*	*5*
12	20	0	1	22	1	2
	8	*0*	*1*	*13*	*1*	*1*
13	9	0	1	3	1	0
	6	*1*	*0*	*5*	*0*	*0*
Total	136	15	11	98	5	9
	70	*4*	*15*	*68*	*5*	*14*

*The upper figure in each block refers to white children, the lower (italicized) figure to Negro children.

Table VI presents findings collected in the study of the 340 white and Negro children comprising the 4-, 5-, and 6-year age groups. Both sexes were represented. Of the total number of 1,780 noncarious deciduous molars studied in them, 106 showed fissures (54 in the first deciduous molar; 52 in the second deciduous molar). It can hardly be said that these figures are unimportant,

for they supply a reliable index of the incidence of this defect at the time of the eruption of these teeth. That fissures do not as a matter of course become carious can be demonstrated by a study of the findings accumulated in the children forming the 10-, 11-, 12-, and 13-year age groups. In these contingents 29 noncarious first deciduous molars and 49 noncarious second deciduous molars showed this defect. It should be noted that no transition to caries of these teeth occurred during a period of from 8 to 11 years. It becomes clear from this and other experiences that fissures are not the important factor in caries (Table VII).

SUMMARY

The fissure incidence in the first and second deciduous molars of a group of 2,211 white and Negro children was ascertained. An analysis of the findings shows that only a very small percentage of the deciduous molars have this defect. The most essential fact which emerges from this investigation is that in no way can the incidence of this fault be compared with that of caries. To imply a direct relationship between the two is definitely erroneous. Other factors must be responsible for the initiation and prevalence of caries in these teeth.

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PHYSIOLOGICAL TOOTH MIGRATION AND ITS SIGNIFICANCE FOR THE DEVELOPMENT OF OCCLUSION

II. The Biogenesis of Accessional Dentition

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THE "accessional dentition"* makes its appearance with the eruption of the first permanent molars which are guided into position by the distal surfaces of the second deciduous molars (terminal plane). When the distal surface of the mandibular molar is mesial to that of the maxillary, thereby forming a terminal plane with a mesial step, no adjusting mechanism is necessary to assure eruption of the first permanent molars into proper cuspal interdigitation (Fig. 1, *A*). If the terminal plane remains perpendicular, an end-to-end relationship of the first permanent molars may result (Fig. 1, *B*).

Three theories have been proposed to explain the occlusal adjustment of the deciduous arches from the straight terminal plane into a mesial step:

A. A mesial forward adjustment of the mandible in its fossa may occur during the period of the deciduous dentition as described by Korkhaus,[4] and Gottlieb and Orban.[3] If the mandible or the lower dental arch move forward, this must result in increased sagittal measurements between distal surface of the lower deciduous canine to the distal of the upper one. In order to determine whether such a movement takes place, this distance between the distal surfaces of the opposing deciduous canines was measured seriatim on plaster reproductions before and after eruption of the permanent first molars.

B. The possibility of a forward migration of the lower teeth during the period of the deciduous dentition was suggested by Zielinsky[14] and others.[2, 6, 8] The observations made in the previous paper of this series[1] showed that the terminal plane remained constant throughout the period of the deciduous dentition. It was therefore necessary to investigate whether such a change in the terminal plane takes place after eruption of the first permanent molars or not.

C. A mesial tipping of the lower first permanent molars after shedding of the lower second deciduous molars may take place as described by Kantorowicz,[5] Strang,[11] and recently reviewed by Speidel.[10] In order to demonstrate any probable movements of the posterior teeth in the upper or lower arches the distance from the distal of the second deciduous molar to the distal of the deciduous canine was measured before and after eruption of the permanent molars.

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*The transformation of the deciduous dentition into the mixed and the permanent dentitions has been divided into two processes: (1) The eruption of the 12 permanent molars, which were not preceded by deciduous teeth, is termed "accessional dentition." (2) The eruption of those permanent teeth which replaced the 20 deciduous teeth are the "successional dentition."

The above subdivision is justified on the basis of the histogenesis. While the "accessional teeth" arise from the (deciduous) general dental lamina, the teeth of the "successional dentition" develop from separate anlage laminae.

RESULTS

A comparative study of casts of 60 cases before and after the eruption of the permanent molars revealed three distinct kinds of normal molar adjustment:

1. In deciduous dentitions which terminated *with a marked mesial step* the first permanent molars erupted immediately into normal cuspal interdigitation without altering the position of the neighboring teeth (Fig. 2, *A*). The occurrence of a mesial step at the end of a deciduous dentition was generally due to an increased mesiodistal measurement of the upper second deciduous molar over that of the lower. This, however, was found only in 8 cases (14 per cent). The mesial axial inclination of the lower first molars as compared with the distal axial inclination of the upper favored their normal occlusal relationship.

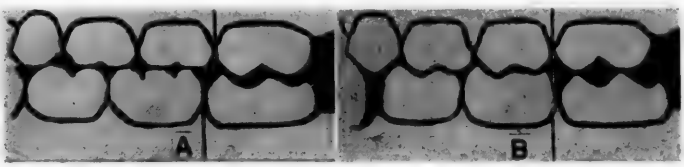
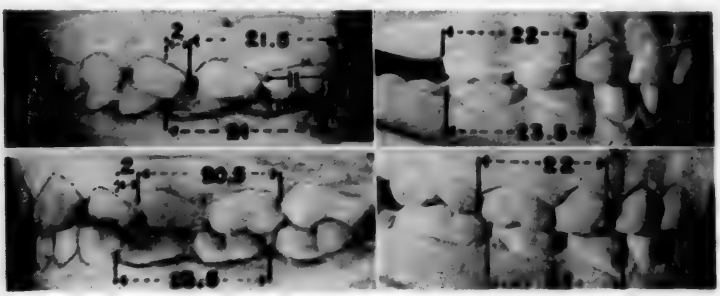


Fig. 1.—The development of occlusion of the first permanent molars.

A, Correct cuspal interdigitation behind a deciduous dentition in which the distal surfaces of the second deciduous molars form a mesial step.

B, End-to-end relationship behind a straight terminal plane of the deciduous dentition.



A. *B.*

Fig. 2.—Illustrations of two kinds of adjusting mechanisms which immediately produce proper molar occlusion.

A, Occlusion of the first permanent molars behind a *deciduous dentition terminating in a mesial step* is achieved directly. (The upper second deciduous molars show mesiodistally the same measurement as the lower.) Case 11.

B, Occlusion of the first permanent molar behind a *straight terminating deciduous dentition of Type I* is effected through mesial migration of the mandibular deciduous molars into the primate space. Case 33.

2. In spaced deciduous dentitions of *Type I with a straight terminal plane* it was observed that following the eruption of the first permanent molars, the straight terminal plane had developed into a mesial step (Fig. 2, *B*). After the eruption of the first permanent molars, measurements of the distance between the distal surfaces of the second deciduous molars and the distal surface of the canines in the mandibular arches showed in all these cases a marked decrease of 1 to 2 mm. A simultaneous closure of the lower primate space was concomitant with this process. The deciduous molars had moved into the primate space. The extent of their mesial migration corresponded with the width of that space.

Fig. 3 shows that the lower first permanent molar moves mesially after its eruption. It first contacts the second deciduous molar and then pushes the deciduous molars into the primate space.

It is apparent that this mesial migration caused the change of the straight terminal plane into a mesial step since the corresponding maxillary distance remained unchanged. This process enabled the upper permanent molars to erupt directly into normal occlusion. Fig. 4 illustrates the progress of these changes in the same dentition of a patient between 5 and 7 years of age.

Fig. 3—Biomechanism of physiologic mesial migration in Case 3. The lower first permanent molar has erupted at 6 years of age. Note a space between molar and second deciduous molar. This space and the primate space have disappeared one year later at 7 years of age.

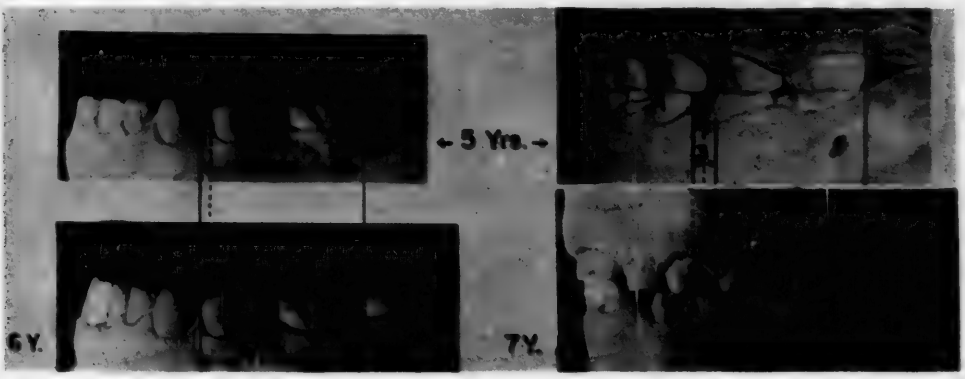


Fig. 4.—Occlusal adjustment of the first permanent molars in Case 60. Between 5 and 6 years of age the distance between the distal of the lower deciduous canine and the postlacteon (pl) decreased 1.5 mm. concomitantly with the closure of the mandibular primate space. The straight terminal plane at 5 years is transformed into a mesial step at 7 years, while the anteroposterior relationship of the deciduous canines remained unchanged. (Note closure of the maxillary primate space upon eruption of the upper central incisor.)

Measurements between the distal surfaces of the opposing deciduous canines revealed that there was no change in that dimension in any of the 60 cases. This is illustrated in Figs. 2, 4, 7, 8, and 9 which show that the occlusal relationship of the canines remained uniformly constant after the alteration of the terminal plane.

3. In closed dentitions of *Type II* a mesial migration of the lower deciduous molars cannot occur because of the absence of the primate spaces. The opposing permanent molars, therefore, are forced to erupt behind a straight terminal plane producing an end-to-end occlusion (Fig. 5).

It is a consistent observation that the permanent first molars move mesially whenever a space is created anteriorly due to loss of contact as the result of caries, extraction, or physiological shedding of a tooth. This indicates that a pressure in a mesial direction persists as long as the process of eruption of accessional teeth goes on. Since the mandibular premolars are narrower than their deciduous predecessors, some space is provided when the lower second deciduous molars are shed. The subsequent mesial migration of the lower permanent first molars change their end-to-end relationship into a normal one. This is the last adjusting mechanism of the permanent molar occlusion.

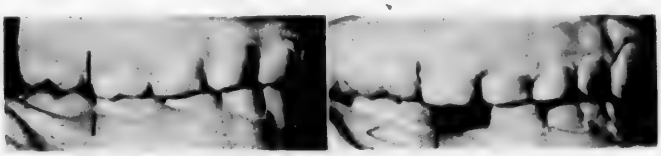


Fig. 5.—After loss of mandibular second deciduous molar the end-to-end relation of the permanent molars behind a *straight, terminating deciduous dentition of Type II* is adjusted by mesial migration of the lower first permanent molars. Case 34.

Fig. 6.—A case which developed permanent *distoclusion*. At 5 years of age the deciduous denture shows a distal terminal step, spacing of the maxillary posteriors and edge-to-edge bite of the deciduous canines.

At 7 years a mesial migration of the upper first molar into the spaces between the upper deciduous posteriors occurred. This resulted in an increased distal step and distoclusion of the permanent molars.

Serial measurements in the maxilla between the distal of the second deciduous molars and distal of the canine revealed that an identical forward pressure also results in the closing of spaces whenever they existed. This is illustrated with models of Case 11 (Fig. 2, *A*) where a space between the upper deciduous canine and first deciduous molar was closed after eruption of the permanent molars. A pronounced mesial migration of the maxillary teeth, particularly when it is absent in the mandible, invariably produces malocclusion of the permanent molars, i.e., distoclusion (Fig. 6).*

The following analysis suggests that the etiological factors of the entire adjusting mechanism are mainly hereditary and endogenous; exogenous factors like function and attrition seem to be of comparatively little importance. The influence of forced mastication on the development of the dentition was studied

*Angle Class II malocclusion also becomes fixed after eruption of the permanent molars behind deciduous dentitions with a terminal plane forming a distal stop. It was observed that here the upper permanent first molar always erupted before the lower one, which may be a helpful indication for the early recognition of this malocclusion.

on serial models for 9 cases with an end-to-end bite and marked cusp attrition of the deciduous molars and canines. Cases of this kind are supposed to produce ideal occlusion.[2, 4, 8] Instead of developing into perfect dentures, malocclusion resulted in 7 of them. Four developed a distoclusion (Fig. 7) and 3 a mesioclusion (Fig. 8).

Simultaneously, the unchanged relationship between the opposing deciduous canines revealed that the malocclusion was not the result of an anterior or posterior shift of the mandible. It was not surprising to note on other serial models that in spite of the absence of heavy attrition and the existence of a pronounced overbite, the permanent molars erupted into a perfectly normal occlusal relationship (Fig. 9).

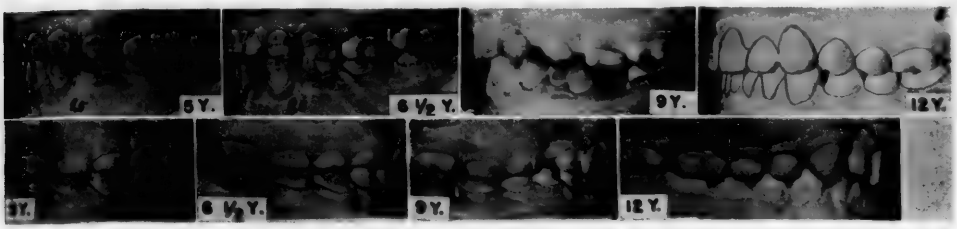


Fig. 7.—Transition of two deciduous dentures with end-to-end occlusion and marked abrasion *into distoclusion* after eruption of permanent molars. No change in the relationship of opposing deciduous canines took place.
A, Case 5. *B*, Case 18.

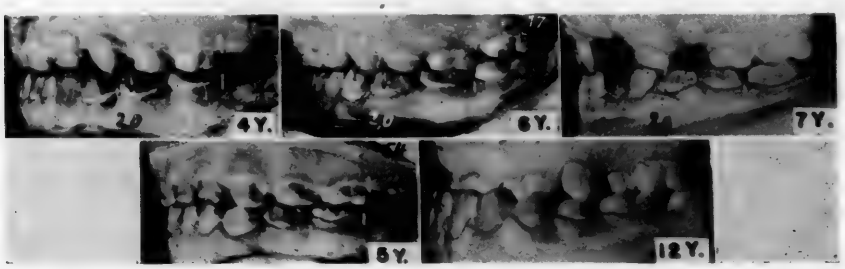


Fig. 8.—Transition of two deciduous dentures from *orthognathy* into *mesioclusion* after eruption of permanent molars. Note constancy of canine relationship.
A, Case 17. *B*, Case 54.

DISCUSSION

Measurements showing unchanged anteroposterior relationship of the opposing deciduous canines reveal that the concepts of a mesial shift of the entire mandible, or of the entire lower dental arch, are not borne out by facts. This is further substantiated by the statement of Thompson and Brodie[12] that the angular relation to the cranium is not altered during development. Sillman[9] also reported in his studies made on serial models from birth to the age of 10 that, "In cases where the child has the potentiality of developing 'good' occlusion, little change is noted in the anteroposterior relationship of the dental arches due to premature loss of deciduous teeth." Therefore, migration of the deciduous molars and not forward shift of the mandible constitutes a physiological measure by which proper occlusion of the permanent molars and mixed dentition is secured.

The cause of the migration may be explained by the pressure exerted by the eruption and root formation of the permanent molars.* It constitutes a physiological regulator of the development of occlusion. The quality of occlusion depends on the extent of the forces and where and when they may become effective.

On the basis of clinical studies three different mechanisms of normal occlusal adjustment have been described: (1) the direct occurrence of a mesial step, (2) the early mesial shift of the lower first permanent molar effecting a closure of primate spaces, and (3) the late mesial shift of the lower permanent molar subsequent to shedding of the deciduous molars. These mechanisms are interpreted as an expression of the law of variation which governs the course of every physiologic process. It is possible to have a combination of two or more of the processes occurring consecutively to produce proper occlusal relationship. The significance lies in the chronological order of their effectiveness.

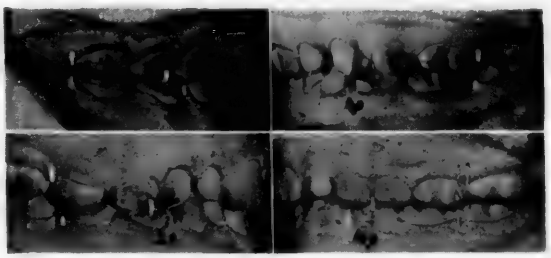


Fig. 9.—Proper occlusion of first permanent molars is achieved in spite of servere overbite and absence of heavy abrasion in the deciduous dentition.
A, Case 2. *B*, Case 3.

The first and second adjusting mechanisms secure immediately proper occlusal relationship of the first permanent molars directly after their full eruption. These are the surest forms of occlusal adjustment. They can be relied upon to maintain proper cuspal interdigitation even after the premature loss of the deciduous molars. The combination of the first and second mechanisms seems to be the uniform pattern in apes. The third is a typical human pattern. It expresses the effort necessary to ultimately attain proper molar occlusion in the phylogenetically reduced jaws of modern man. The second and third kinds are often combined when the closure of the primate space initiates an insufficient cuspal interdigitation of the first molars. The third mechanism thus secures normal occlusion.

The widely held concept of the inhibition of normal molar adjustment by a severe incisal overbite could not be confirmed in this study. Serial observations revealed that deciduous dentitions with gliding articulation had the potentiality to produce poor molar occlusion while some dentures with deep overbite developed normal molar occlusion.

*This early mesial migration is not to be mistaken for that minute one described by Weinmann and Sicher,[12] which they described as the result of the occlusal forces, and which is believed to be continuous.

The practical application of these findings to the problems of clinical orthodontia emphasizes the importance of changing an end-to-end occlusion of the first molars into their proper cuspal interdigitation as early as possible.

SUMMARY

A further clinical study of the development of occlusion in 60 children by means of serial plaster reproductions of the dental arches was made. Through comparative measurements, three different biologic mechanisms of normal occlusal adjustment of the accessional teeth were found:

1. The occurrence of a terminal plane forming a mesial step in the deciduous denture allowed the first permanent molars to erupt *directly* into proper occlusion without altering the position of the neighboring teeth.

2. The presence of a mandibular primate space and a straight terminal plane was conducive to proper molar occlusion by means of an *early* mesial shift of the mandibular deciduous molars into the primate space upon eruption of the lower first permanent molar.

3. Closed deciduous arches and a straight terminal plane resulted in a transitory end-to-end relationship of the first permanent molars. Proper occlusion was effected through a *late mesial shift* of the mandibular permanent first molars subsequent to the shedding of the deciduous second molars.

Migration of the lower deciduous and permanent molars constituted the physiological measure to secure proper occlusion of the permanent molars. There was no mesial shift of the mandible as indicated by the unchanged anteroposterior relationship of the opposing deciduous cuspids. Function and attrition had less bearing on the molar adjustment than widely assumed. The anatomical pattern of the deciduous arches controlled the ability of the deciduous and permanent molars to migrate mesially as a result of the eruptive force of the accessional teeth.

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PHYSIOLOGICAL TOOTH MIGRATION AND ITS SIGNIFICANCE FOR THE DEVELOPMENT OF OCCLUSION

III. The Biogenesis of the Successional Dentition

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THE REPLACEMENT of the deciduous incisors by the permanent ones inaugurates the "successional dentition." Tooth measurements as given by Wetzel[14] indicate that all lower permanent incisors are on an average +3.8 mm. and the upper +5.5 mm. wider than their deciduous predecessors. The statistical values recently calculated by Seipel[11] show an average increase in width of +5.0 mm. for the lower and +7.0 mm. for the upper permanent incisors. Therefore, in order to accommodate harmoniously the larger permanent incisors a physiological expansion of the arches is necessary. Some room is provided by the interdental spaces and thus closed deciduous dental arches need a greater enlargement than the spaced arches.* The physiological expansion must be sufficient to avoid crowding which is the most frequent malformation of modern dental arches. Its prognosis and prevention is therefore one of the very important requirements in orthodontics.

The expansion of the arches transversally and sagitally is described in practically all textbooks as "physiological spacing" which takes place between 4 and 6 years of age. It has been previously shown[1] that such changes do not occur in the deciduous dental arches. However, two different morphological types of deciduous arches were recognized, one continuously spaced (Type I) and one continuously closed (Type II).

The present study deals with the changes which affect the dental arches during and after eruption of the permanent incisors and follows in general the approach of Korkhaus and Neumann's serial investigations.[8] Lewis,[9] Cohen,[5] Goldstein and Stanton,[7] and Sillmann[13] have noted the changes in width and length of the arches at regular intervals. In this study they are designated according to the stage of development of the dentition, namely: Stage 1—The deciduous dentition is completed. Stage 2—The permanent first incisors have erupted. Stage 3—All the permanent incisors have erupted. Measurements in width of the arches are taken between the deciduous canines and between the second deciduous molars at the gingival margin.[1] An attempt is made to establish some practical conclusions in regard to the differences in development which exist in the arches of Type I and Type II.

A variation of opinion exists in respect to the time and degree of the forward extension of the dental arches. Most investigators[4, 6, 7, 8, 15] have used the distal of the deciduous second molars (postlacteon) as the fixed points from

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*The mean sum of spaces, according to Seipel, is 2.7 mm. for the lower arches and 3.8 mm. for the upper arches.

which to measure the forward growth of the arches. Franke[6] calculated a retraction of 3.7 mm. of the anterior portion of the lower arch after eruption of all the successional teeth. Previous findings,[2] however, showed that the postlactea move mesially, especially in Type I arches, when the lower first permanent molars erupt. These landmarks therefore become unreliable. The mesiodistal relationship of the upper to the lower deciduous canines, however, was found to remain constant even after mesial migration of the lower deciduous molars. Forward movement of the prosthion and infradentale is measured from the center of a line connecting the distal contact points of the deciduous canines to these anterior points (Fig. 1). This distance represents the length of the frontal portion of the arch; the difference in length in serial models, taken before and after eruption of the permanent incisors, indicates the extent of forward growth of the alveolar process.

The increase in labiolingual thickness of the upper permanent incisors over their deciduous predecessors has been believed to be greater than the increase in thickness of the lower permanent incisors over their deciduous homologues. This has been credited to an apparent increase in forward extension of the upper arch.[8, 15] Since no values for the labiolingual thickness of the deciduous incisors are available in the literature, this dimension is measured in both deciduous and permanent incisors.

RESULTS

The study material consists of serial plaster reproductions of the dentitions of 60 children taken at different developmental stages.

TABLE I

AVERAGE INCREASE IN WIDTH OF THE MANDIBULAR DENTAL ARCHES DURING AND AFTER ERUPTION OF PERMANENT INCISORS

		INTERCANINE WIDTH					DISTANCE M_2-M_2			
		PRESENT STUDIES			KORKHAUS		PRESENT STUDIES		KORKHAUS	
TYPE	STAGE	NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.
I	1-2	15		0.5	2	1.25	15	0.3	2	0.75
	1-3	24	0/3.0	*2.27*	2	2.0	23	1.7	2	1.75
II	1-2	5		1.2	3	1.2	4	1.0	3	0.2
	1-3	15	0/5.0	*2.5*	16	2.4	10	1.0	10	0.75

Type I: Deciduous dentitions with spacing.
Type II: Deciduous dentitions without spaces.
Stage 1: Deciduous dentition is completed.
Stage 2: First permanent incisors have erupted.
Stage 3: All permanent incisors have erupted.

Table I shows the mean increase in *width of the mandibular arches* after the eruption of the permanent incisors. Between Stages 1 and 2 an increment in intercanine width of 0.5 mm. is noted in Type I and 1.2 mm. in Type II arches. After eruption of all the lower incisors (Stage 3) a mean increase in intercanine width of 2.2 mm. occurs in cases of Type I and 2.5 mm. in Type II arches. The maximum increase of 5.0 mm. is found in Stage 3 of Type II. The width of the

arches between the deciduous second molars is also increased in both types; Type II, however, yields lower values than Type I in Stage 3.

A comparison of the results with those obtained by Korkhaus and Neumann[8] shows a close conformity.

In regard to the rate of lateral growth, the strongest growth impulse in the mandible is noted during the eruption of the permanent second incisors or stage 3. It was observed that first incisors which erupted in a slightly rotated position became straightened when the second incisors began to erupt. This is illustrated in Fig. 1 of the plaster reproductions of Case 32 taken of the child at 6 and 7½ years of age. This case is representative for the development of the 44 deciduous dentitions of Type I. They were all succeeded by mixed dentures with properly aligned incisors.

The lower arch of Case 42 (Fig. 2) demonstrates a favorable development of a Type II arch with crowded deciduous anteriors. A compensatory widening of 4.0 mm. after eruption of the permanent first incisors made up for the early lack of sufficient lateral growth. Only 9 out of 16 cases of Type II, however, produced such properly aligned anteriors. The rest of the cases (43 per cent) developed crowding.

The comparatively greater effort to produce harmoniously arranged permanent anteriors in previously closed than in spaced arches is shown in Fig. 3, *a* and *b* of Type I cases, and *c* and *d* of Type II cases. The mean increment of 2.5 mm. in width was increased when the permanent incisors entered directly into the spaces of their predecessors. They immediately established contact with adjacent teeth. These erupting teeth exerted a lateral force displacing neighboring teeth thus widening the intercanine distance as much as 5.0 mm. If, however, the permanent second incisors erupted lingually or in torsiversion to the first incisors, a lateral displacement could not be effected (Fig. 3, *e, f, g*).

TABLE II

SECONDARY SPACING BETWEEN THE MAXILLARY DECIDUOUS ANTERIORS AFTER ERUPTION OF MANDIBULAR PERMANENT FIRST INCISORS

(Increase in intercanine width of the L[lower] and U[upper] A[arches] in mm.)

	CASE 1		CASE 32		CASE 33		CASE 40		CASE 42		CASE 56	
PERIOD	L.A.	U.A.	L.A.	U.A.	L.A.	U.A.	L.A.	U.A.	L.A.	U.A.	L.A.	U.A.
O-A	0	0	0	0	0	0	0	0	–0.5	0	–	–
A-B	1.5*	*0.5*	0.5*	*0*	0.5*	*0.3**	1.0	*1.0*	2.0	*1.0*	1.5*	*1.5**

Period O-A: Deciduous dentition between 4-6 years.
Period A-B: Mixed dentition 6-7 years { Upper arch: 4 deciduous incisors; Lower arch: 1-1 erupted
*M_1 erupted before lower permanent first incisor.

This mechanism disclosed the importance of the initial arrangement of the permanent tooth germs in the jaw, which may be governed by endogenous factors, independent of exogenous influences. Furthermore, as stated by Moore,[10] the etiology of crowding is at least partly based upon hereditary factors.

Table II demonstrates the increment in *intercanine width* of both arches of some selected cases during the interval between the full eruption of the lower permanent first incisors until the shedding of the upper deciduous first incisors.

This represents a period of 6 to 9 months. It is found that the upper arch, which still consists of all its deciduous teeth, responds to the enlargement of the lower arch. The eruption of the lower permanent first incisors produces a

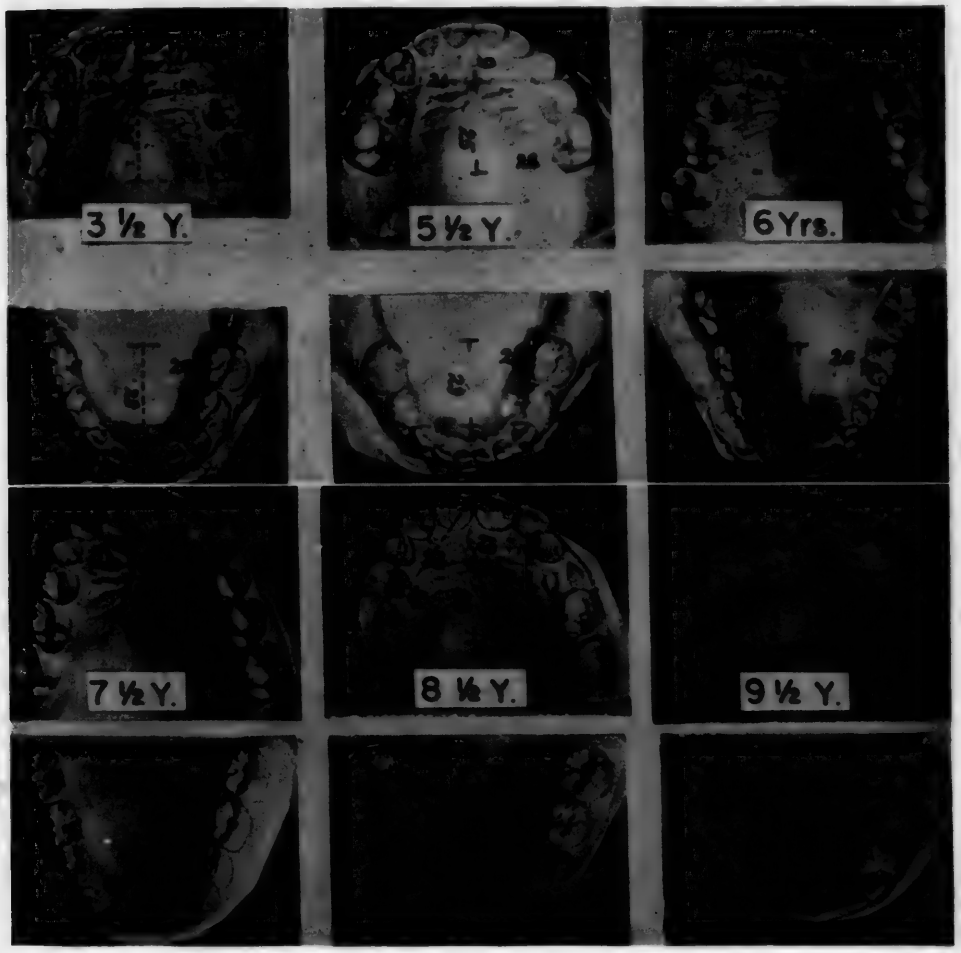

Fig. 1.—Development in Case 32, representative for deciduous dentitions of Type I.
Between 3½ and 5½ years no changes in dimensions of the deciduous arches occurred; the postmolar areas appeared.

Between 5½ and 7½ years note 2.0 mm. intercanine widening of both archs, 4.0 forward extention of the upper arch and 2.0 mm. of the lower arch.

Between 8½ and 9½ years the tending for crowding of the mandibular incisors upon eruption of the permanent canines was arrested by extraction of the lower deciduous second molars.

simultaneous widening of the maxillary intercanine distance of about half of the mandibular expansion. If this value exceeds more than 1 mm., a separating of the upper deciduous anteriors becomes apparent, that is, a so-called secondary spacing. This can be seen in Case 42 between 5 and 6 years of age (Fig. 2) and in Case 40 (Fig. 2, *C* of a previous paper[1]).

TABLE III

AVERAGE INCREASE IN WIDTH OF MAXILLARY DENTAL ARCHES DURING AND AFTER ERUPTION OF UPPER PERMANENT INCISORS

		INTERCANINE WIDTH					DISTANCE m_2-m_2			
		PRESENT STUDIES			KORKHAUS		PRESENT STUDIES		KORKHAUS	
TYPE	STAGE	NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.	NO. CASES	AVERAGE INCREASE MM.
I	1-2	11		1.4	4	2.3	13	0.5	4	0.37
	1-3	24	0/4.0	*2.5*	2	3.0	19	1.5	2	2.0
II	1-2	3		1.5	15	2.1	5	0.7	19	0.6
	1-3	12	0/6.5	*3.2*	12	3.7	8	1.6	16	1.5

Table III gives the average figures of increment in *width of the maxillary arches* following the eruption of the permanent incisors. At Stage 2 the increase in intercanine width is approximately equal in both types of arches, namely, 1.5 mm. At Stage 3 the mean increase in width amounts to 2.5 mm. in arches of Type I, and 3.2 mm. in those of Type II.

In Type I and Type II arches the mean increase in width between the second deciduous molars in the upper arch is about the same for both Stages 2 and 3 and amounts to about one-half of the increase in intercanine width. The greatest impulse of lateral growth occurs in the maxillary arch contrary to that in the mandible at Stage 2. Apparently this occurs to provide room for the permanent first incisors, which are about 4.3 mm.[11] wider mesiodistally than their deciduous predecessors.

In the Type I cases it is observed that all the upper spaces between the deciduous anteriors become closed upon eruption of the permanent first incisors (Fig. 4, Baume[2]). If enough lateral growth took place, a transitory or permanent spacing recurred between the permanent incisors. This is illustrated by Case 42, the child at 8 years of age (Fig. 2). The same phenomenon in the lower arches can be seen in Fig. 3, *a* and *b*. Nevertheless the Type II cases yielded higher mean values, 40 per cent developed crowding of the permanent incisors.

TABLE IV

AVERAGE FORWARD EXTENSION OF FRONTAL PORTION OF ARCHES DURING AND AFTER ERUPTION OF PERMANENT INCISORS

(Increase of the frontal arch length from a line connecting the two distal contact points of the deciduous canines to the pr or id)

		LOWER ARCH			UPPER ARCH		
TYPE	STAGE	NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE INCREASE MM.	NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE INCREASE MM.
I	1-3	26	0/3.0	1.3	36	–0.5/4.0	2.2
II	1-3	16	–0.5/3.0	1.3	12	0/4.0	2.2
I & II	1-2	16		0.5	7		1.5
	1-3	42	–0.5/3.0	1.3	48	–0.5/4.0	2.2

Table IV reveals that the mean *forward extention* of the lower arches is 0.5 mm. and that of the upper arches is 1.5 mm. after eruption of the permanent first incisors. This is equal for both Types I and II. No correlation is seen

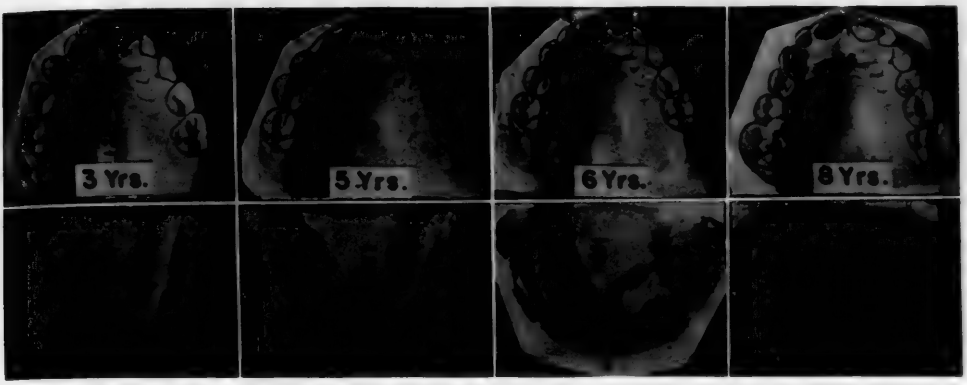

Fig. 2.—Series of development in Case 42 which has a mandibular arch of Type II and a maxillary arch of Type I.

Between 3 and 5 years note decrement in length of deciduous arches and the severely crowded lower incisors.

Between 6 and 8 years note the secondary spacing of the upper deciduous anteriors upon eruption of the permanent lower first incisors.

Between 5 and 6 years note proper alignment of permanent lower incisors by physiological intercanine widening of 4.0 mm. (After extraction of first deciduous molars, space maintainers have been worn.)

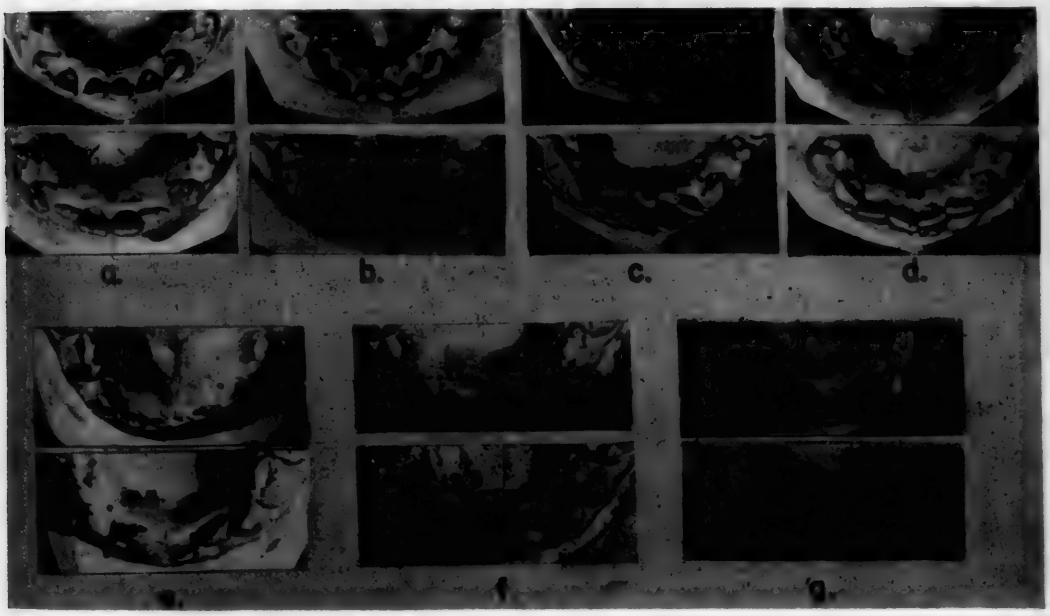

Fig. 3.—Lateral and forward growth during exchange of lower incisors in 7 cases.

a, Case 35, Type I }
b, Case 7, Type I } Note + 2.0 mm. lateral growth and + 1.0 mm. forward growth.

c, Case 2, Type II. Note + 2.0 mm. lateral growth and + 1.5 mm. forward growth. The permanent anteriors are slightly crowded.

d, Case 31, Type II. Note maximum expansion of 5.0 mm. laterally and 3.0 mm. anteriorly; nevertheless slight crowding of permanent anteriors occurred.

e, Case 29, Type II }
f, Case 40, Type II } Lingual eruption of the permanent second incisors prevented a lateral displacement of the canines and produced severe crowding.

g, Self-correction of crowding 2 months after extraction of deciduous canines.

between the amount of forward growth and the presence or absence of spaces. After eruption of all the permanent incisors the forward extention of the lower arches averages 1.3 mm. and the upper arches 2.2 mm. The maximum deviations are 3.0 mm. and 4.0 mm. Thus a difference of 1.0 mm. is noted between the mean forward extentions of the upper and lower arches.

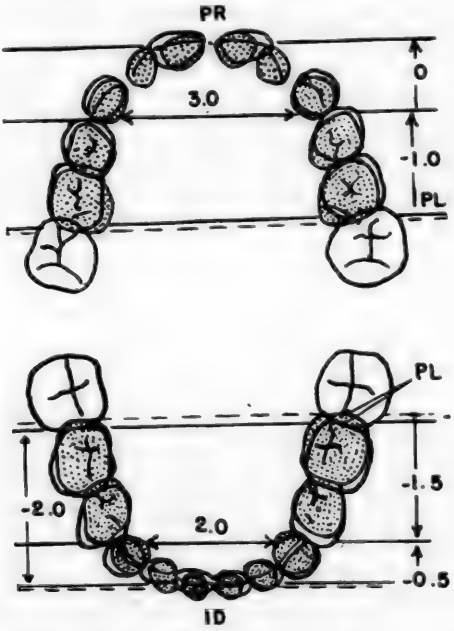

Fig. 4.—Diagraphic tracings of Case 8, child at 5 years (stippled area) and 8 years superimposed on a line connecting the two distal contact points of the deciduous canines. Note shortening of the frontal portion of the lower dental arch by –0.5 mm.* and mesial migration of lower postlacteon (*PL*) by –1.5 mm.*

*The numbers represent the differences in dimensions in mm.

With the aid of the stereograph of Schwarz[12] tracings of the arches of the same individual at two consecutive stages were made and superimposed on the canine axis. Fig. 4 shows the graphs of Case 8, the child at 5 and 8 years of age. It exhibits a backward movement of the infradentale (*ID*) by –0.5 mm. and

TABLE V

AVERAGE LABIOLINGUAL THICKNESS OF FIRST DECIDUOUS INCISOR AND ITS PERMANENT SUCCESSOR

(Measured on models in mm.)

	MANDIBULAR				MAXILLARY			
	PRESENT STUDIES			MÜHLREITER AVERAGE MM.	PRESENT STUDIES			MÜHLREITER AVERAGE MM.
FIRST INCISOR	NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE THICKNESS MM.		NO. CASES	MIN.-MAX. VARIATION MM.	AVERAGE THICKNESS MM.	
Deciduous I.	42	3.2/4.5	3.9	–	50	4.5/6.0	5.0	–
Permanent 1	43	4.5/6.5	5.6	5.9	38	6.0/8.0	6.9	7.3
Difference	I-1		*1.7*				*1.9*	

no positional change of the prosthion (*P3*). The important role of forward growth of the anterior portion to secure proper alignment of the anteriors is illustrated in Fig. 1 for the upper arches and in Fig. 3, *d* for the lower arches.

Measurements in Table V of the *labiolingual thickness* of the deciduous first incisors and their permanent successors indicate a mean increment of 1.7 mm. between the mandibular homologues and 1.9 mm. between the maxillary ones. Thus the increase in thickness of the permanent first incisors is about equal in both jaws.

DISCUSSION

The transition from the deciduous to the successional dentition was accompanied by little change in the dimensions of the arches.* Various factors account for proper arrangement of the permanent incisors:

1. *Alignment of the Mandibular Permanent Incisors.*—Concurring with the findings of Lewis[9] and Korkhaus and Neumann,[8] this study revealed that at the time of the eruption of the permanent incisors there occurred a transverse widening of the mandibular arches. This represents one of the physiologic processes to provide space for the erupting permanent incisors and their greater mesiodistal widths.

The mean increment of 2.3 mm. (the maximum deviation being 5.0 mm.) may be of practical value in suggesting the biologic limits of therapeutic widening of the mandibular arch. The widening of the arches seems to be due to lateral growth of the alveolar process and not alone to a displacement of neighboring teeth. This is based on the fact that the anterior section did increase in width in cases of Type I even when sufficient space existed to accommodate the permanent incisors. The result was temporary or permanent spacing of the permanent incisors. Some lateral growth occurred in the posterior region simultaneously with lateral growth in the canine region. This is evidenced by the increase in the distance between the second deciduous molars.

In the postnatal development, intensified lateral growth of the alveolar processes were found to occur during the following periods: (1) the formation of the deciduous arches following the period of lactation; (2) the eruption of the permanent incisors, and (3) the eruption of the permanent canines and premolars, which is shown later.

A review of the data reveals that the crowding of anteriors is due to a lack of growth of the alveolar processes. The deficiency in alveolar growth, contrary to earlier contentions, can occur in the first period of growth during the formation of the deciduous arches. Then it is manifested in an absence of spaces and frequently in slightly crowded deciduous anteriors (Type II). There exists, however, a compensatory mechanism which, during the time of eruption of the permanent incisors, sometimes makes up for this early lack of sufficient growth.

In regard to the significance of spaces in the deciduous dental arches, the following observations were made:

*The maximum amount was found to be 6.5 mm. while the simultaneous growth of the other parts of the head did go on at a higher rate.[3]

(A) In Type II, the mean increase in intercanine width was greater than in Type I, thus compensating for the absence of spaces. This was substantiated by the fact that a greater number of maximum values were recorded in Type II than in Type I.

(B) All the spaced deciduous arches produced properly aligned permanent anteriors.

(C) Crowding of the anteriors was found in almost half of the cases of Type II at Stage 3, in spite of the relatively greater mean increment in width. Previously spaced deciduous dental arches produced favorable alignment of their permanent anteriors whereas those arches without spaces tended to produce crowded anteriors.

2. *Alignment of the Maxillary Permanent Incisors.*—In the maxilla a similar biologic mechanism prevailed as in the mandible. Observations of serial models, however, revealed two distinct modifications.

The first of these modifications is caused through the eruption sequence. The permanent first incisors erupt in the maxilla 6 to 9 months later than in the mandible. Due to expansion of the lower arch, in some cases a secondary spacing of the upper deciduous anteriors occurred. Contrary to accepted views, this spreading did not appear in the completed deciduous dentition but at the beginning of the successional dentition when the still undeveloped maxilla was adapted to the widened mandible upon eruption of the permanent lower first incisors. Lewis's[9b] illustration of a physiological spacing seems to be based upon this circumstance.

A second modification is due to morphology and concerns the maxillary primate spaces described above.[1] These frequent diastemata between the upper deciduous second incisors and canines provide space for the wider permanent first incisors.

The initial arrangement of the tooth germs in the maxilla as well as in the mandible also plays an important role in the final arrangement of the teeth. The germs of the permanent incisors are mainly found in two positional forms:

(A) The permanent first incisors are in labioversion to the second incisors. The proper alignment occurs as the result of the biologic lateral growth; if this is absent, linguoversion of the laterals persists which in the maxillary arch seldom corrects itself because of the articulation with the mandibular teeth.

(B) The germs are in torsiversion, singly, or in pairs. These teeth will not straighten out after eruption if there is a lack of alveolar growth. This was experienced with a case where, after extraction of the deciduous canines, the rotated permanent second incisors became harmoniously aligned.

The clinical application of these findings emphasizes the importance of early therapy. Irregular arrangement of the incisors often can be averted with minimum active expansion of the arches before the roots of the permanent incisors are fully formed.

3. *Forward Growth of the Alveolar Processes.*—In view of the limited lateral growth, it was apparent that there must exist another biologic process to allow for a harmonious alignment of the wider permanent incisors. This consisted in a forward extension of the dental arches in direct connection with the

eruption of the permanent teeth. This extension was found to be greater in the maxilla by 1.0 mm. than in the mandible, but was not analogous to the greater increase in width of the maxilla over the mandible. The increase in thickness of the permanent first incisors over their deciduous homologues was similar. Thus it is obvious that the smaller forward extention of the lower arches cannot be due to tooth morphology. This difference therefore may be interpreted as a lesser growth of the mandibular alveolar process in the sagittal direction. Paleontologists[12, 15] have pointed out that there is an evolutionary tendency for the human mandible to undergo a reduction. Present data may furnish some facts for this contention.

The measurements of forward growth explain the so-called shortening of the lower deciduous (lacteal) arch after replacement of the anteriors.[6] Taking into account the mesial migration of the lower first permanent molars, a maximum shortening of –0.5 mm. was noted in only 2 cases out of 60. The lower permanent incisors erupted in a limited linguoversion and remained unchanged due to a lack of forward development of the arch.

According to Weinmann and Sicher,[16] forward as well as lateral growth appear histologically as circumferential and periosteal bone apposition on the labial and buccal alveolar surfaces. A histological study of the course of this process would be advisable, since the present study gives evidence of only a limited forward extent of the dental arches; this occurred at the time of the eruption of the successional teeth.

SUMMARY

A comparative and biometrical study of serial casts of 60 cases before, during, and after eruption of the permanent incisors demonstrated the following findings:

1. Expansion of the dental arches in the anterior region, to accommodate the larger successional incisors into proper alignment was brought about by a lateral and frontal alveolar growth during the time of the eruption of these teeth.

2. The mean increase in intercanine width was greater in the upper arches than in the lower ones, and again greater in previously closed upper or lower deciduous arches than in previously spaced ones.

3. In the mandibular arches the strongest impulse of lateral growth was noted during the eruption of the second incisors, in the maxillary arches during the eruption of the first incisors.

4. An occasional "secondary" spacing of the upper deciduous anteriors occurred when the still undeveloped maxillary arch was somewhat widened upon eruption of the permanent lower first incisors.

5. Spaced deciduous arches generally produced favorable alignment of the permanent incisors while about 40 per cent of the arches without spaces produced crowded anteriors.

6. The average amount of forward extension of the arches showed no difference between previously spaced and closed arches but was 1.0 mm. greater in the upper arches than in the lower ones.

7. According to the tooth measurements the difference between the maxillary and mandibular forward extension could not be credited to a greater increase in thickness of the crowns of the upper permanent incisors over that of the lower ones. It was interpreted as an expression of an evolutionary tendency toward a lessened forward growth of the mandibular alveolar process.

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THE EFFECT OF PYRIDOXINE DEFICIENCY ON THE JAWS OF MICE

A. Periodontal Structures

B. Mandibular Condyle

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PYRIDOXINE evidently plays a role in protein metabolism. Rats placed on a high protein (30 to 45 per cent) pyridoxine-deficient diet develop skin lesions sooner and die in a shorter time than those whose pyridoxine deficient diets contain lower amounts (15 per cent) of protein.[1] Histological studies of lesions of the skin and other organs have been made in rats and pigs fed pyridoxine-deficient diets.[2] The long bones of mice[3] have been studied in both high protein (54 per cent) and lower protein (18 per cent) pyridoxine-deficient diets.

The present investigation deals with the histological changes seen in the periodontal structures and mandibular condyle of mice maintained on high and lower protein pyridoxine-deficient diets for various periods of time.

MATERIALS AND METHODS

Twenty-eight mice of strain C_{57} black, 28 days of age, were used in these experiments. The animals were divided into two groups of fourteen mice each (equal sex ratio). Group I was fed ad libitum the usual synthetic diet deficient in pyridoxine, while Group II was placed on a synthetic diet deficient in pyridoxine but containing 54 per cent casein. The composition of the rations was as follows:

	Group I	Group II
Sucrose	68.0 Gm.	30.0 Gm.
Vitamin test casein	18.0 Gm.	54.0 Gm.
Vegetable oil	10.0 Gm.	10.0 Gm.
U.S.P. Salt Mix No. 12	4.0 Gm.	4.0 Gm.
Cod liver oil	1 c.c.	1 c.c.
Riboflavin	0.5 mg.	0.5 mg.
Thiamin	0.2 mg.	0.2 mg.
Nicotinic acid	1.0 mg.	1.0 mg.
Choline HCl	1.0 mg.	1.0 mg.
Inositol	100.0 mg.	100.0 mg.
Calcium pantothenate	10.0 mg.	10.0 mg.
Biotin	25.0 mg.	25.0 mg.

Water was available at all times.

Animals were maintained on the deficient diets for one, two, four, or eight weeks. At the end of each experimental time period, mice were sacrificed, the

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A. *B.* *C.*

Fig. 1.—*A*, Two-week synthetic control; *B*, Two-week deficient, normal protein. Note inflammatory cells in tip of epithelium of interdental papilla. Alveolar crest is lower than normal; *C*, Two-week deficient, high protein.

A. *B.* *C.*

Fig. 2.—*A*, Eight-week synthetic control; *B*, Eight-week deficient, normal protein; *C*, Eight-week deficient high protein.

heads fixed in 10 per cent formalin, and the mandibular joint and jaws decalcified and embedded in paraffin as previously described.[4] Serial sections were prepared for study on all tissue blocks. Animals of corresponding strain and age maintained on Diet I plus 0.2 mg. per cent pyridoxine served as normal controls.

RESULTS

Periodontal Structures

Group 1.—The periodontal structures of the one-week deficient animals are normal in appearance.

After two weeks on the deficient diet, the epithelium of the interdental papillae shows a polymorphonuclear leukocytic infiltration at the very tip of the epithelium. There is no evidence of either new bone formation or resorption of the interdental bone. The transeptal fibers are attached at the cementoenamel junction and are normal. In some areas of the surface of the interdental septum of bone there is a thin basophilic-staining line (Fig. 1). Four-week deficient animals show little additional change. The crest of the alveolar bone has receded from the surface epithelium which is still infiltrated by polymorphonuclear leukocytes.

After eight weeks on the deficient diet there is further recession of the alveolar bone. In some areas of the periodontal membrane there is hyalinization of the connective tissue fibers. At the apical areas of the tooth, some of the periodontal fibers stain basophilic. The epithelium of the interdental papillae is atrophic. There is still the infiltration of polymorphonuclear leukocytes at the tip of the interdental papillae. The transeptal fibers are still attached at the cementoenamel junction. There is no evidence of active osteoclastic resorption of bone nor resumption of osteogenesis (Fig. 2).

Group 2.—After one week on the deficient diet there is no change in the periodontal tissues.

Animals deficient two weeks show a cessation of growth of the interdental alveolar bone and there is a rather wide margin of basophilic-staining material on its surface. The bone marrow appears to contain more adult red blood cells than is normal. The transeptal fibers are no longer attached at or near the cementoenamel junction, but attach further apically. The epithelium which covers the interdental papillae contains several polymorphonuclear leukocytes not only at the very tip but also closer to the basement membrane. The basement layer is distorted and frayed. The lamina propria in many areas of the section shows changes ascribed to edema.

After four weeks on the deficient diet the changes are much more marked. The interseptal alveolar bone has receded considerably from its original height. The transeptal fibers are attached much further apically than formerly, and the epithelium which covers the interdental papillae is destroyed in many areas and is replaced by a mass of finely granular basophilic-staining material and polymorphonuclear leukocytes. The epithelial attachment is now below the level of the cementoenamel junction. There is an increased vascularity of the periodontal membrane. There is atrophy of the bone marrow. In some

areas of the interdental septa there is resorption of the alveolar bone. The resorption lacunae are deeper than normal. The destruction of oral epithelium, which shows hydropic degeneration and necrosis, is well exemplified in areas posterior to the third molar, or between the second and third molar.

After eight weeks on the deficient diet there are further changes (Fig. 2). The attachment of the periodontal tissues to the teeth now almost reaches the crest of the alveolar bone so that there are very few intact transverse fibers. The alveolar crest fibers are relaxed. The epithelium of the interdental papillae is completely replaced by polymorphonuclear leukocytes and necrotic debris. The epithelium elsewhere shows continued degenerative changes. In some areas of the periodontium behind the third molar and elsewhere there are small areas of calcified material which appear much like fragments of cementum or bone.

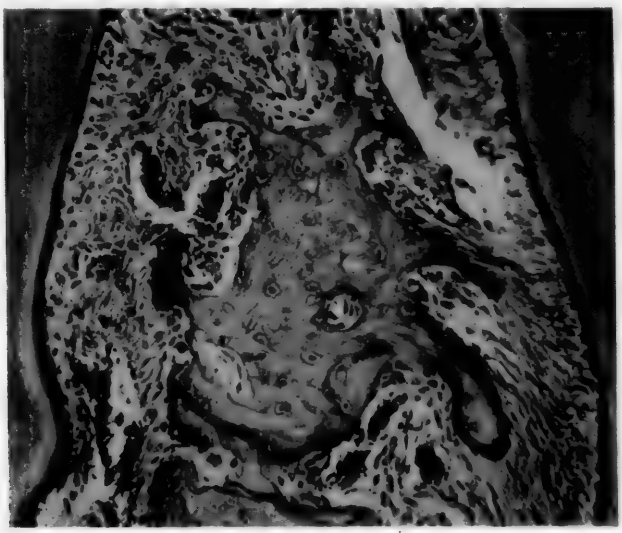


Fig. 3.—Eight-week deficient, high protein. Area between second and third mandibular molar. Note spicules of bone extending to support periodontal fibers.

There are dilated capillaries in the marrow spaces as well as in the periodontal tissues, most of which are engorged by red blood cells. There is considerable evidence of vascular resorption of the alveolar bone. This occurs particularly in the area between the second and third molar. Here spicules of bone extending to support the periodontium are found (Fig. 3). Around these spicules there is a good deal of resorption, these resorbed areas being replaced by rather loose fibrous connective tissue and many dilated capillaries. In some areas where the internal structure of the alveolar bone is resorbed it is replaced by large, round, and polyhedral shaped cells the cytoplasm of which is extremely granular.

Condyle

Group 1.—After one week on the deficient diet there is slight narrowing of the width of the cartilage cap; while after two weeks on the deficient diet,

the cartilage cap is noticeably narrower than usual. The cells of the hypertrophic zone are quite distinct but the cells of the intermediate zone are not as numerous as usual. There is a hyalinization and a calcification of the matrix in the hypertrophic zone. The trabeculae of bone which is linked beneath this cartilage is normal and is lined by large polygonal osteoblasts (Fig. 4).

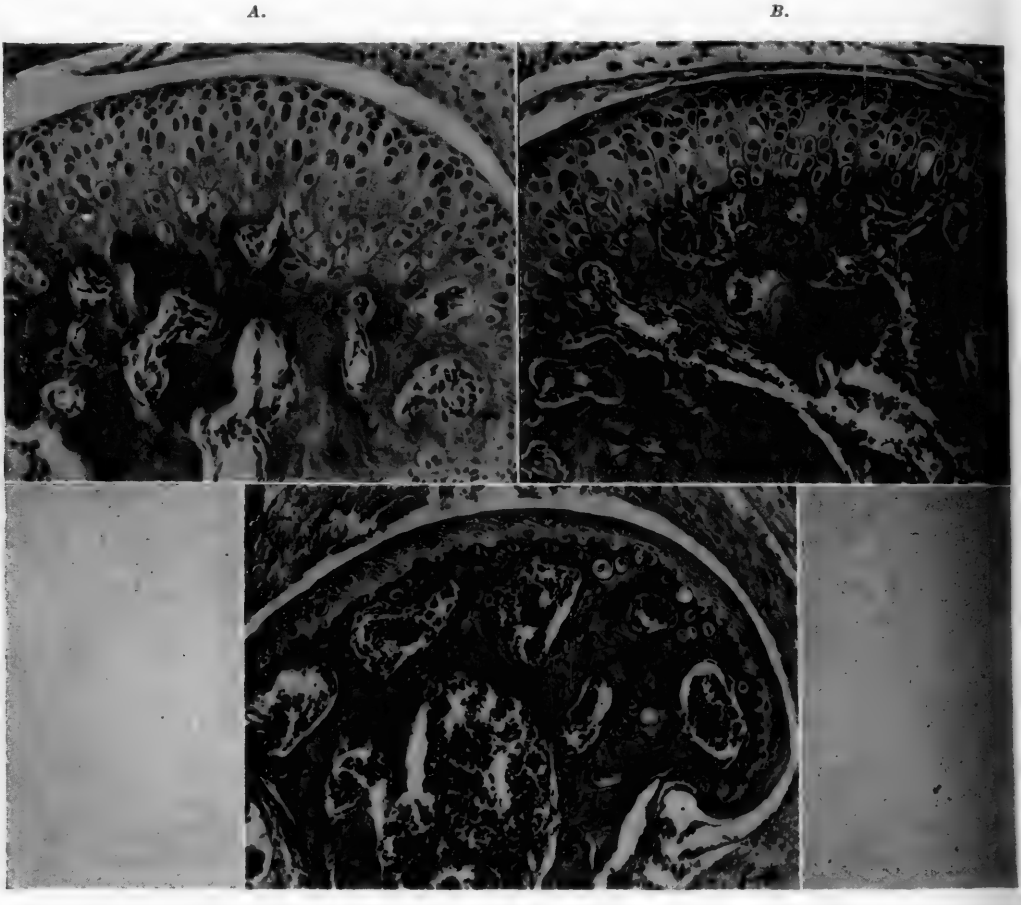


Fig. 4.—*A*, Condyle of two-week synthetic control animal; *B*, Two-week deficient, normal protein; *C*, Two-week deficient, high protein.

In animals deficient for four weeks, there are marked atrophic changes in the cartilage cap. There are no cells in the intermediate zone and very few hypertrophic cells. There is a complete or almost complete transverse bony linkage beneath the cartilage. The chondrocytes are much smaller than usual. The flat, undifferentiated cells are normal in appearance and in amount. In some areas the marrow carrying capillaries and osteoblasts have almost reached this zone of undifferentiated cells (Fig. 5). The osteoblasts which are reduced in size are small and spindle shaped. The marrow is atrophic.

Eight-week deficient animals show a well-calcified cartilage matrix. There is a complete or almost complete bony linkage beneath the cartilage, but the undifferentiated cell layer is still present and normal. The marrow is atrophic. The trabeculae which are present are covered by an occasional very thin, spindle-shaped osetoblast. The osteocytes are normal in appearance (Fig. 6).

Group 2.—After one week on the deficient diet there is some slight narrowing of the cartilage cap due to a reduction in the number of hypertrophic cells. The subcartilage area, that is, the zone of erosion, is still quite active, although the osteoblasts are reduced in size and number (Fig. 7).

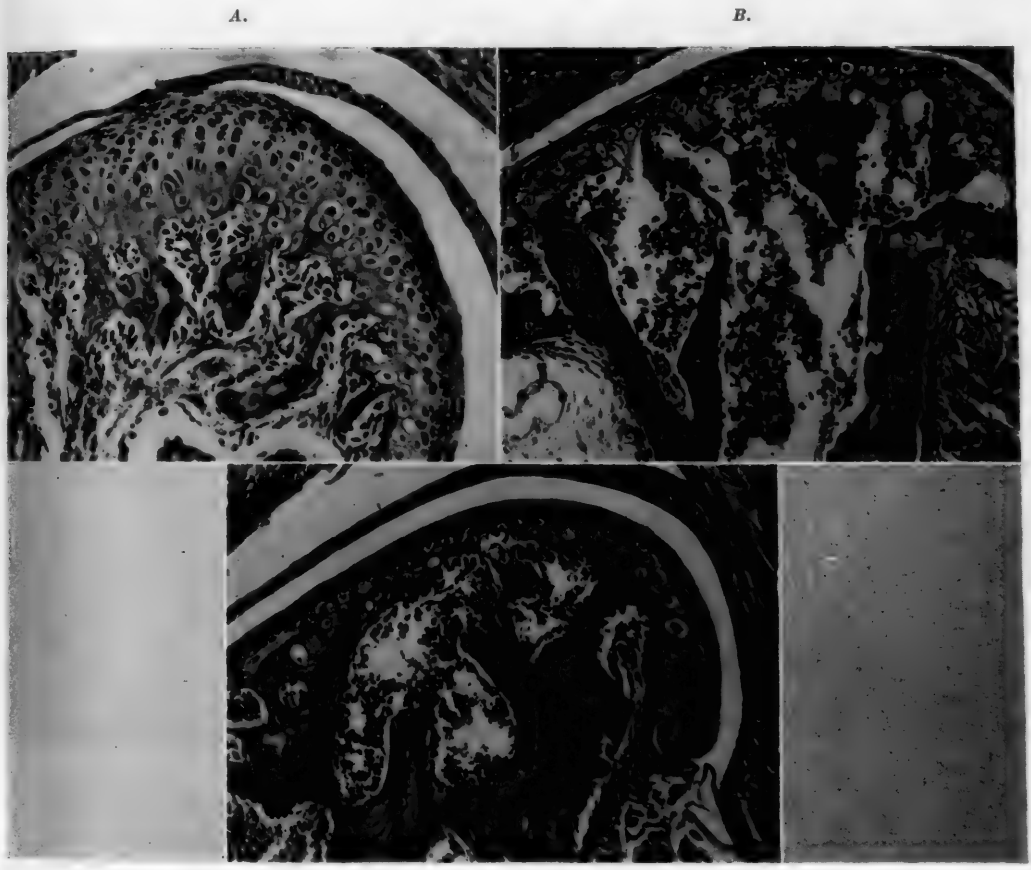
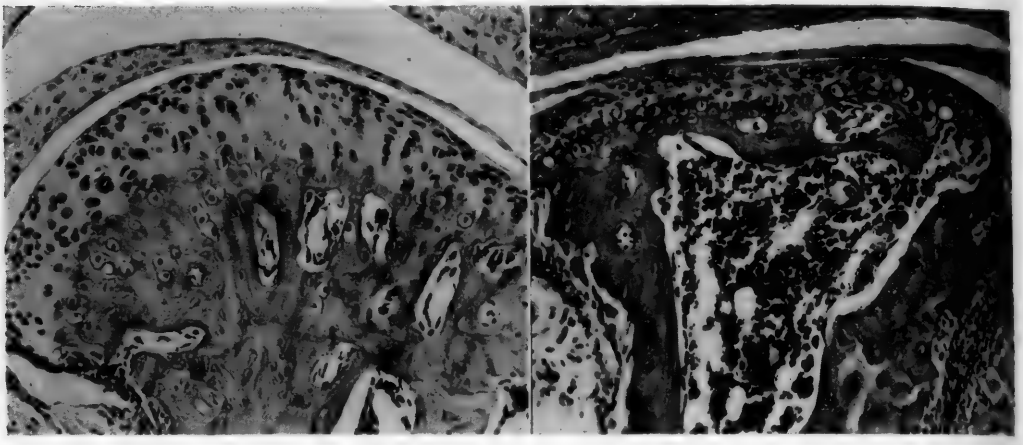


Fig. 5.—*A*, Four-week synthetic control; *B*, Four-week deficient, normal protein; *C*, Four-week deficient, high protein.

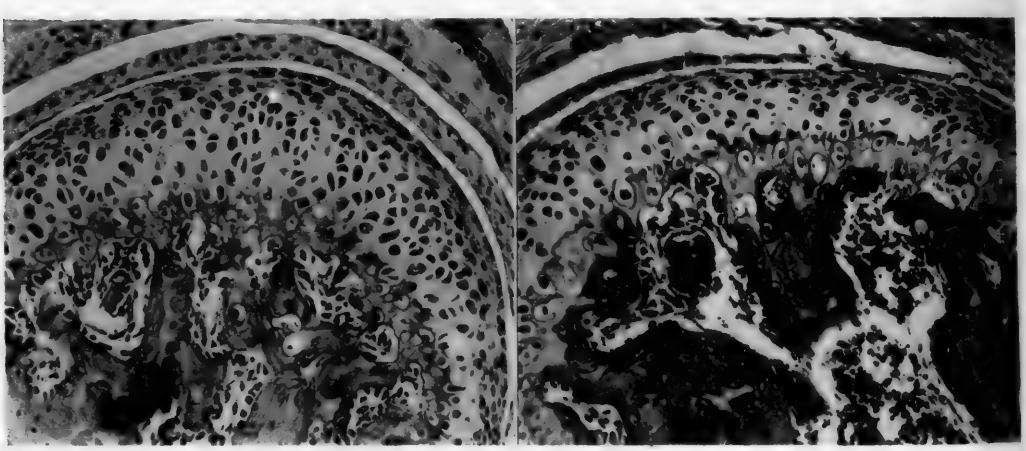
Two-week deficient animals show a marked narrowing of the cartilage cap with early subcartilage linkage. However, the capillaries in the zone of erosion are well filled and carry osteoblasts to the hypertrophic zone. This zone shows

some calcification of the matrix. The flat resting or undifferentiated cells are normal in number and in size. Most of the chrondocytes are smaller in size than is normal (Fig. 4).



A. *B.*

Fig. 6.—*A*, Eight-week synthetic control; *B*, Eight-week deicient, normal protein.



A. *B.*

Fig. 7.—*A*, One-week synthetic control; *B*, One-week deficient, high protein.

After four weeks on the deficient diet there is a marked decrease in activity as is indicated by subepiphyseal bony linkage. The chondrocytes are atrophic and diminished in size as well as in number, and there is atrophy of marrow. However, there is a zone of erosion which is still vascularized. The osteoblasts which line the bony spicules are smaller than normal (Fig. 5).

In the eight-week deficient animals there is marked atrophy of the cartilage cap as well as atrophy of the bone marrow. There is bony linkage separating the cartilage from the marrow space. The chondrocytes are atrophic.

DISCUSSION

Pyridoxine plays a part in the metabolism of protein, probably because it is converted into codecarboxylase and functions as the co-enzyme of several amino acid decarboxylases. There is also some interrelationship between pyridoxine and the unsaturated fatty acids.[5] In view of these facts, a discussion of the mechanism by which pyridoxine deficiency affects tooth and bone growth could not possibly be made at this time. Too little is known of the role of protein and fat in bone and tooth development.

The findings are consistent with previous information, however. Those animals which received pyridoxine-deficient diets high in protein showed changes earlier than those whose deficient diet contained "normal" amounts of protein. Animals maintained on high protein pyridoxine-deficient diets showed more marked destructive and inflammatory changes than animals maintained on an 18 per cent casein pyridoxine-deficient diet. The changes in this vitamin B deficiency differ from those seen in pantothenic acid deficiency[4] in that inflammatory changes are marked with pyridoxine deficiency while they are characteristically absent in pantothenic acid deficiency.

SUMMARY AND CONCLUSION

Animals maintained on pyridoxine-deficient diets show a cessation of growth of the mandibular condyle, regressive changes in the alveolar bone, and ulceration of the epithelium of the interdental papillae, this epithelium being replaced by acute and chronic inflammatory cells and necrotic debris. The addition of large amounts of protein to the deficient diets causes these changes to occur earlier and to appear more severe.

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THE EFFECT OF X-RAY IRRADIATION ON THE DEVELOPMENT OF THE MANDIBULAR JOINT OF THE MOUSE

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THIS study was undertaken to observe the response of the mandibular joint of the mouse to varying doses of x-ray irradiation. A survey of the literature has indicated that such a study has not been previously reported. Several investigators have observed the effect of x-ray irradiation upon the epiphyseal growth of long bones.[1, 2, 3] Their findings indicate that epiphyseal cartilage exhibits a marked radiosensitivity.

Systematic histologic studies of the growth of the mandibular joint of normal mice have been undertaken by Levy.[4] The growth and development of the mandibular condyle exhibit both intramembranous and intracartilaginous bone formation. The process differs from the development of long bones in that there is only one primary center of ossification. Moreover, the condylar cartilage constitutes the essential growth center of the mandible.

METHODS

Thirty-eight Swiss and C_{57} black mice 2 to 7 days of age were irradiated in the area of the mandibular condyle. Littermates and littermate controls were used whenever feasible. All animals were maintained on Purina Laboratory Chow with water available at all times.

A Phillips Metalix Contact x-ray machine was used under the following conditions: K.V. 50, M.A. 2, t.s.d. 20 mm., 0 filter. (The H.V.L. is 0.3 mm. Al.) The diameter of the field to be irradiated was regulated by the use of ports, the openings of which varied from 5.0 to 7.0 mm. in diameter. The three dosages and approximate exposure times which were used were 1,500 r (18½ seconds), 3,000 r (38 seconds), and 5,000 r (62 seconds).

The recorded dosages represent the intensity of radiation at the skin surface only and do not represent the intensity at the underlying bone structure. Since the intensity of radiation falls off rapidly at a depth of several millimeters, the opposite condyle was compared with the one receiving the heavier dosage.

Following irradiation, twenty-four of the mice which survived were sacrificed at time intervals from five to sixty-two days. Littermate controls were also sacrificed to coincide with these intervals. The weights and sex of both irradiated and control animals were recorded. The heads of mice under 3 weeks of age were fixed and decalcified in Bouin's solution (with 10 per cent acetic acid). The heads of the older mice were split longitudinally and fixed in 10 per cent formalin. All tissues were decalcified, embedded in paraffin, sectioned in a frontal plane, and stained with hematoxylin and eosin.

RESULTS

Gross Observations.—Erythema and epilation of the irradiated skin surface occurred approximately five days following irradiation. With 3,000 to 5,000 r there was a necrosis of the skin and underlying tissue. Administration of 1,500 r to animals under 4 days of age produced a hemiatrophy in the mandible

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which became prominent after several weeks. This type of change is not as prominent with 3,000 and 5,000 r.

Microscopic Observations.—Seven days following irradiation (5,000 r) the growth cartilage of the condyle is damaged. The capillaries are dilated, numerous extravasated red cells are present in the narrow spaces, and there is an aplasia of the marrow. The resting or undifferentiated cells at the articular surface are normal in number and appearance. There is a marked reduction in the number of cells of the intermediate zone and of the hypertrophic cells. The nuclei of the hypertrophic cells are indistinct and there is an irregular distribution of chromatin material in the cytoplasm. In some areas bone is in contact with the cartilage with no intervening marrow space. The interarticular disc is slightly thickened and the cranial portion of the joint exhibits a slight marrow aplasia.

Fig. 1.—Condyle of normal 12-day-old mouse. *A*, Interarticular disc; *B*, undifferentiated or embryonic zone; *C*, intermediate zone; *D*, hypertrophic zone; *E*, zone of erosion. (Low ×.)

The changes visible at nine and ten days postirradiation (5,000 r) are similar to those at seven days with the exception that the marrow aplasia is more prominent (Figs. 2 and 3). The periosteum of the condyle is thin. The remaining hypertrophic cartilage cells are enlarged. The nuclei exhibit varying degrees of karyorrhexis, karyolysis, and pyknosis, and the cytoplasm is finely granular. In some areas there is a subcartilage linkage to the bone trabeculae. The trabecular bone is poorly developed, stains more basophilic than normal, and the cement lines are prominent. In Howship's lacunae beneath the periosteum are multinucleated giant cells. These giant cells are also present in the marrow spaces. Fibroblastic activity is present, and in several animals there is hemorrhage into the marrow spaces and joint capsules. New bone formation is not evidenced and there is a relative increase in the number of heterophil leukocytes in the marrow spaces.

With dosages of 1,500 and 3,000 r the damage to the intermediate and hypertrophic zones is not prominent as compared with the damage resulting

from 5,000 r (Fig. 4). With 1,500 r, the fibrosis and aplasia of the marrow is minimal.

Twelve days postirradiation (5,000 r) there is a gradual increase in the relative number of erythroblasts. The intermediate and hypertrophic zones of cartilage are thin and a subcartilage linkage to the bone is still present. Osteoblastic activity is slight.

Fig. 2.—Condyle of 11-day-old mouse nine days following irradiation (5,000 r). Note the marrow aplasia and poor trabecular development. (Low ×.)

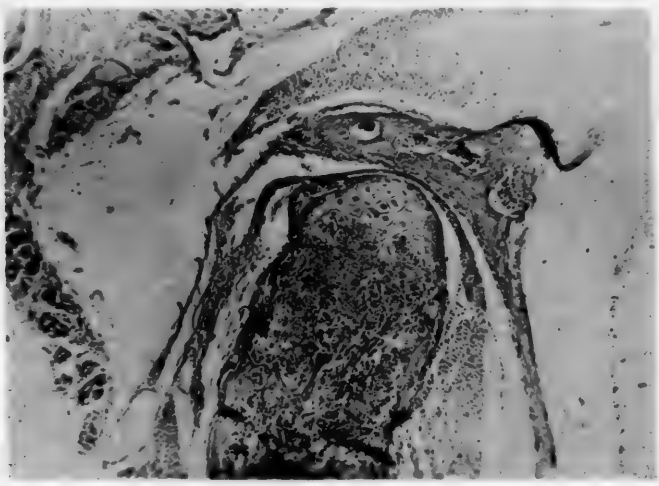


Fig. 3.—Condyle of 11-day-old mouse nine days following irradiation (5,000 r). The marrow spaces contain extravasated erythrocytes and degenerating marrow cells. The nuclei of the hypertrophic zone are pyknotic. (Low ×.)

Fifteen to eighteen days following irradiation (5,000 r) there is a marked fibrosis of the condylar bone marrow (Fig. 5 *A*). Heterophil leukocytes and fibroblasts represent the predominating cell types. Osteoblastic activity is greater than in earlier postirradiation stages, but fewer osteoblasts are present than in the normal controls. The intermediate and hypertrophic cartilage zones

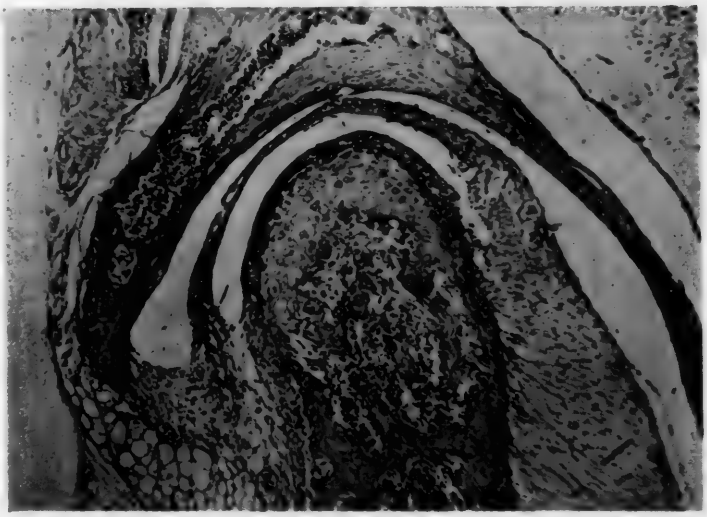
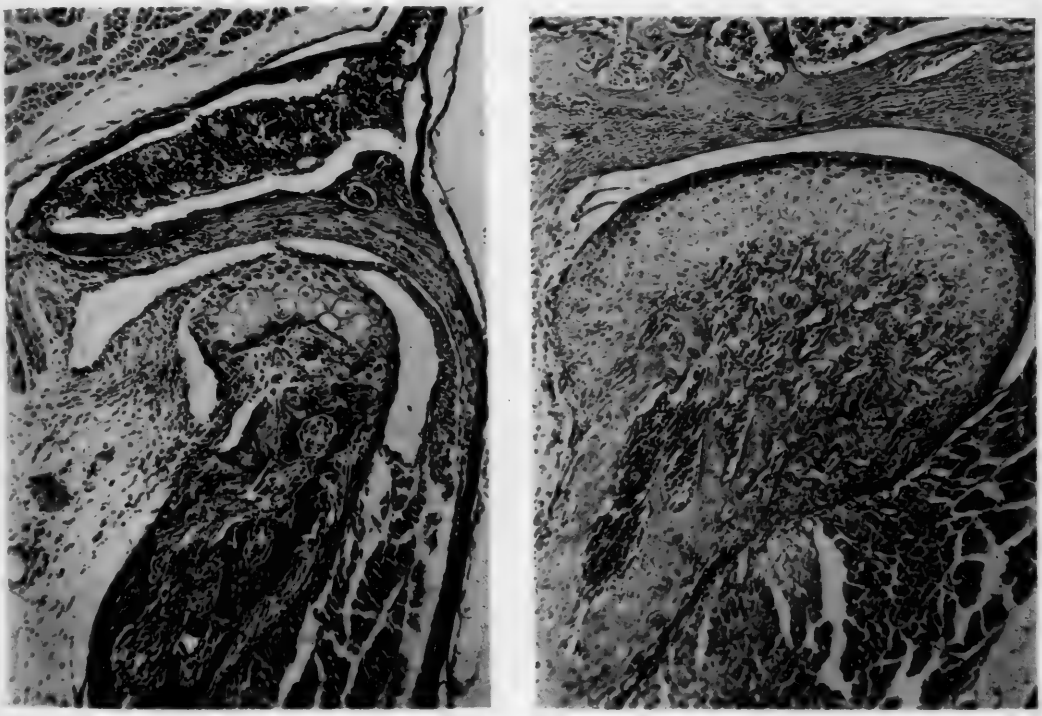


Fig. 4.—Condyle of 10-day-old mouse seven days following irradiation (1,500 r). There is a distortion of the cartilage but the destruction is minimal as compared to the 5,000 r series. (×75.)



A. *B.*

Fig. 5*A*.—Condyle of 22-day-old mouse eighteen days following irradiation (5,000 r). Note the abnormal hypertrophic cells and irregular trabeculae. There is a marked fibrosis and aplasia of the marrow. (×75.)

Fig. 5*B*.—Condyle of normal 22-day-old mouse. (×75.)

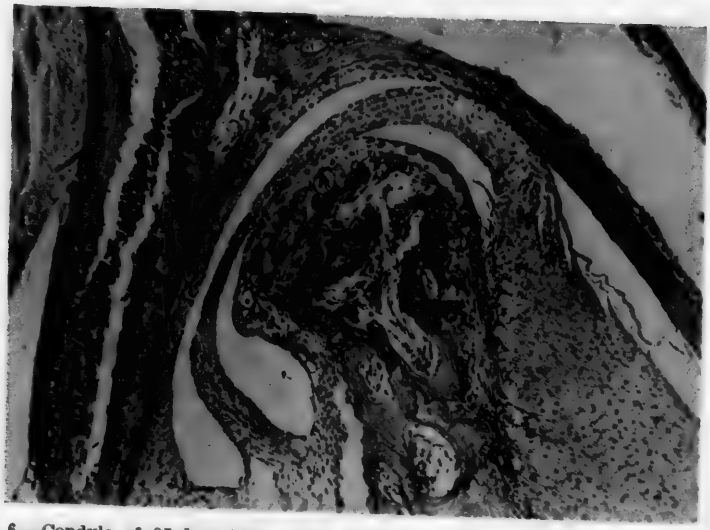
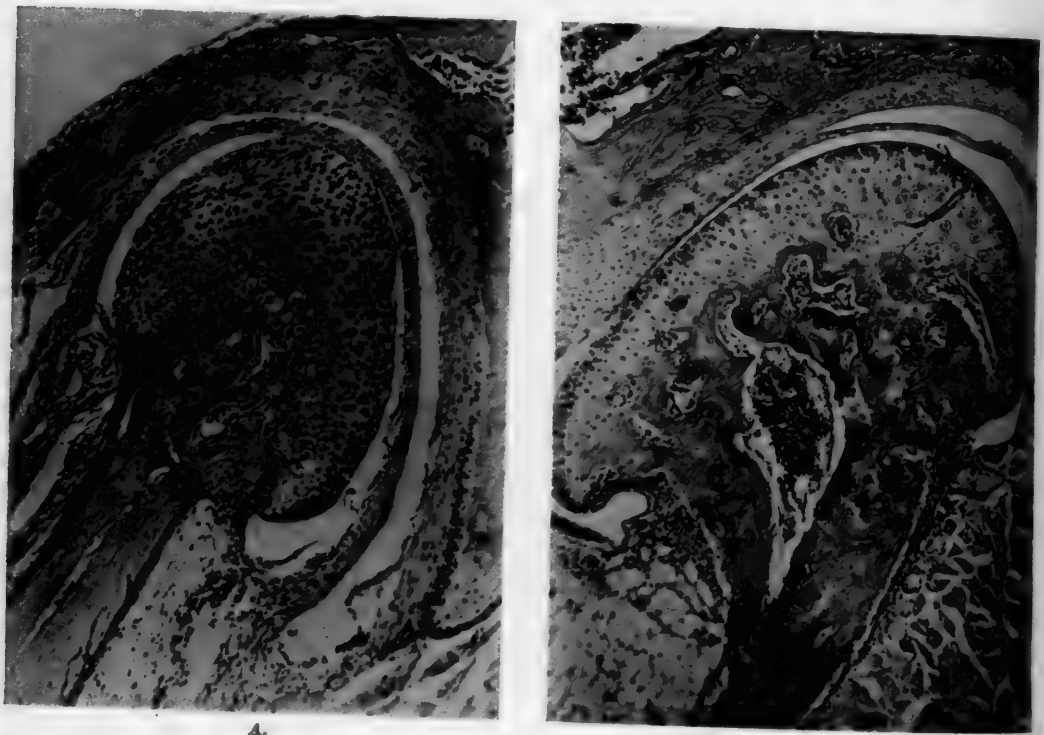


Fig. 6.—Condyle of 27-day-old mouse twenty days following irradiation (5,000 r). The thin cartilage cap and extensive subcartilage linkage are suggestive of changes which normally occur in older animals. (×75.)



A. *B.*

Fig. 7*A*.—Condyle of 64-day-old mouse sixty-two days following irradiation. There is a hyperplasia of the undifferentiated and intermediate zones. (×75.)
Fig. 7*B*.—Condyle of normal 64-day-old mouse. (×75.)

are thinner than normal. The hypertrophic cells are several times normal size and exhibit karolysis. Trabecular bone formation is irregular and the bone exhibits an abnormal basophilia.

Eighteen days postirradiation (1,500 r) the thickness of the cartilage cap and the marrow cellularity are normal. However, there is some distortion of the hypertrophic cells and of the trabecular bone.

Eighteen days postirradiation (3,000 r) the aplasia and fibrosis of the bone marrow is slight. However, irregular trabecular development is prominent. (Fig. 6.)

Twenty-nine days following irradiation (5,000 r) there is a decrease in the fibrotic marrow and an increase in the number of marrow cells and osteoblastic activity. Some of the hypertrophic cells are distorted. The head of the condyle is flat and underdeveloped and the trabeculae are thick and irregular.

The mandibular condyle of the animals thirty-seven days following irradiation (5,000 r) appears normal except for a broadness and flatness. The distortion of trabecular development is similar to that observed 29 days postirradiation.

Sixty-two days postirradiation there is a trabecular consolidation with an almost complete lack of intervening marrow spaces (Fig. 7 *A*). The change is more prominent than in normal controls. There is a marked increase in the number of cells in the undifferentiated and intermediate zones, and there is evidence of active proliferation of these zones. Hyperplasia of the undifferentiated and intermediate zones is seen occasionally in normal animals, but not to this extent.

SUMMARY

X-ray irradiation of the mandibular joint produces a marked inhibition in the process of ossification.

Irradiation of the mandibular condyle of the mouse with 1,500, 3,000 and 5,000 r results in damage to the intermediate and hypertrophic zones with a subsequent marrow aplasia and fibrosis.

The cranial portion of the joint and the interarticular disc are relatively radioresistant.

The growth potential may be restored to some extent approximately six weeks following irradiation (5,000 r).

Sixty-two days following irradiation (5,000 r) there is a marked increase in the cellular activity of the resting zone and the intermediate zone.

The author wishes to express his appreciation to Mrs. Mary Gross, Miss Dorothy Krug, and Mrs. Lois Lunin for technical assistance.

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SALIVARY AMMONIA AND ITS CORRELATION TO DENTAL CALCULUS

I. Ammonia Nitrogen Development From the Supernatant Liquid and the Residue of Centrifuged Saliva During a Nine-Day Incubation Period

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THE ETIOLOGY of dental calculus has long been a challenge to investigators in dental research. Only recently have the organic aspects of saliva composition been investigated in relationship to the formation of calculus. Saliva would seem to be the source of dental calculus since it is normally present in the mouth and constantly bathes the teeth. A search for the organic chemical substances in the saliva of calculus-susceptible individuals, compared with that of calculus-free individuals, should bring this perplexing problem to a clearer understanding.

This study was undertaken to determine if a correlation exists between the concentration and production of ammonia nitrogen in the saliva of calculus-present, calculus-free, and periodontal-diseased individuals. A brief survey of the work that has been done in the past is essential for a better understanding of this problem.

HISTORICAL BACKGROUND

An early explanation for calculus formations suggested that chemical changes occurred in the mouth which reduced the so-called "solvent power" of the saliva for calcium salts. The calcium salts were precipitated onto the tooth surface. Hunter,[1] in 1803, conjectured this viewpoint but gave no explanation as to the cause of the lowered solvent power of the saliva. Burchard,[2] in 1895, reviewed Hunter's work and came to the same conclusion. In 1911, Kirk,[3] stated that the precipitation of calcium salts in the mouth resulted from a loss of carbon dioxide and from the action of ammonia and other basic substances found in the mouth. These basic substances, he thought, came from the decomposition of protein particles which were present in the mouth and in the breath. He further brought out the fact that ammonia would cause a precipitation of calcium phosphate from its acid solution as calcium phosphate or magnesium ammonium phosphate, the so-called triple phosphate.

These phosphate salts have been identified as constituents of certain varieties of calculus. The alkaline earth phosphates must be retained in a state of

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solution in the saliva in some form of acid combination. The acidity can be produced by an excessive amount of dissolved carbon dioxide. Any agency which reduces the acid element tends to form a precipitate of the alkaline salts. Responsible for this action are the escape of carbon dioxide, the presence of ammonia in the breath, or the introduction of some basic substance into the saliva. An example of the latter group was pointed out by Kirk[3] in the betel nut chewers of the Malay Archipelago, where the natives have a liking for lime on their betel nuts and begin chewing this nut at an early age. The lime causes a basic reaction, thus precipitating the calcium and magnesium salts. The teeth of the natives in this section are so fully covered with calculus that, in many cases, they exfoliate from the mouth, an extreme example of calculus formation in the human oral cavity.

During the same period, Talbot[4] believed that the ammonia came from the lungs and as it entered the oral cavity caused a basic reaction of the saliva and led to precipitation of lime salts. An excess of these salts was thought to be present in the blood of calculus-susceptible individuals.

To further clarify the source of ammonia in the mouth, Prinz,[5] in 1921, showed that fresh saliva collected from the duct of one of the salivary glands showed little ammonia. Mixed saliva found in the mouth showed a much greater concentration of ammonia. He explained this finding as being due to protein decomposition which gave ammonia as an end product along with other substances. Frank[6] reported that the ammonia content of saliva was higher in those individuals having a greater tendency to form calculus. He believed the inspired and expired air played no part in contributing ammonia for calculus formation. To Frank, the only remaining possible sources were blood, tissue fluids, and products of local substances in the saliva itself. The ammonia concentration was most constant two or three hours after meals. He found the greatest daily variations in the saliva of persons having the most calculus. He showed that as the saliva stands at room temperature, the ammonia content increases very appreciably. He concluded that ammonia in saliva plays an important role in calculus formation but indicated that the high ammonia concentration was an effect of calculus, rather than a cause of calculus formation.

Not all investigators attributed the source of ammonia to protein degradation or to the presence of calculus. Badanes and Parodneck[7] showed that choline has a close relationship to systemic alkalinity. Individuals with nervous disorders have an increased choline content in their blood, and those with carcinomas usually become neurotic from worry, their blood showing a comparable increase in alkalinity. The authors further pointed out that bacteria which have entered the saliva decompose choline, with ammonia as one of the end products. This ammonia then combines with the carbon dioxide which is present in the saliva, setting free the acid calcium salts leading to the precipitation of these salts in a basic medium.

Bliss[8] found that calculus from the teeth of dogs contains urease. This enzyme converts urea in the saliva into ammonium carbonate, which further breaks down into ammonia, carbon dioxide, and water. The idea that the content of ammonia in saliva is related to calculus as an effect rather than a

cause is thus strengthened. Bliss also found that when the concentration of urea in the saliva was high, as in nephrectomized dogs, enough ammonia was formed by the enzymatic hydrolysis of urea to produce ulceration. The alkalinity of saliva also contributed to the liberation of free ammonia in this experiment. Maupetit,[9] as well as Hench and Aldrich,[10] found high salivary ammonia levels in nephritic individuals.

Another concept of calculus formation was formulated by Prinz.[5] He stated that an increased concentration of salivary colloids on the surface set free calcium salts which then adhered to the sinking colloid material and were thus carried out of solution. Sachs[11] proposed that calcium precipitation occurred in the mouth when organic material came in contact with the unstable mixture of calcium salts. A decade later, Wittkop,[12] as well as Herrenkneecht and Becks,[13] did not agree with this theory, stating that saliva is a stable colloid suspension having positively charged particles.

The formation of calculus resulting from decomposition of saliva was suggested by Fox[14] as far back as 1814. This theory of calculus formation was given credence by the work of Black.[15] He attributed the hardening of calculus to the decomposition of agglutinated colliodal material which he called calcoglobulin.

The association between periodontal disturbances and calculus led Bass and Johns[16] to assume that calculus formation was a result of periodontal disease. Prinz showed that inflammatory exudates tend to form calculus more quickly. Heinemann[17] pointed out that tissue degeneration in any part of the body is frequently associated with calcification and he tried to show its correlation with calculus formation in the oral cavity.

The fact that special secretory glands are present in the mouth and cause deposition of the inorganic salts by the liberation of some element, was first claimed by Serres[18] in 1817. Smith[19] had a similar idea of the formation of calculus. He believed that phosphatase was present in the gingival epithelium. He showed by experimentation that the phosphatase broke down the organic phosphate, thus leading to the formation of inorganic phosphate salts which were later deposited on the teeth.

Another important concept of calculus formation was that bacteria caused its deposition on the teeth. Ficinus[20] in the mid-nineteenth century, believed that the denticolae were responsible for the deposition of calculus. Klebs,[21] in 1876, showed that leptothrical organisms become calcified in natural springs and thus might be responsible for the formation of calculus in the human mouth. However, the organisms referred to by Klebs were found to be saprophytic leptothrices which do not occur in the mouth. After ten years of investigation it was claimed by Naeslund[24] that Actinomyces were the organisms that usually occurred in the mouth to form dental calculus. The validity of Naeslund's work was questioned by Bibby.[25] Naeslund based his classification upon the appearance of the organisms at a magnification of 260 diameters, but by this means it is impossible to distinguish Actinomyces from any other bacillary forms. According to Bibby[25] and Bulleid[26] *Leptotrichia buccalis* is the organism that Naeslund must have studied and is the one found in calculus deposits. In

a series of experiments performed in vitro by Bibby[25] (1934) it was shown that bacteria play some role in fixing the calculus deposits onto the teeth. In the absence of bacteria, all other conditions being optimal, no calculus is deposited. The organisms most predominant in these deposits were the Leptotrichia, and not the Actinomyces, as claimed by Naeslund.

Each of the above-mentioned theories has its fallacies and thus cannot be considered the sole answer to calculus formation. Of all the opinions mentioned concerning the cause of calculus formation, two main groups can be discerned. The first claims that the calcium salts are precipitated from an unstable saliva due to some physical or chemical agency. The second asserts that bacterial action causes some change in the saliva which tends to precipitate the alkaline earth salts.

Because of the recent interest in ammonia nitrogen in relationship to the dental caries problem, and the past interest in ammonia nitrogen as a predisposing factor in calculus formation, this study was undertaken. The relationship between the ammonia nitrogen concentration of the sediment and the supernatant liquid of the centrifuged saliva, and the presence or absence of dental calculus, would give some information concerning the chemistry involved in its formation. It would also show the relationship of the ammonia nitrogen developing ability in the saliva of calculus-forming individuals compared to the saliva of nonformers by allowing the saliva to stagnate in a 37° C. incubator over a period of time. This relationship would show whether the ammonia nitrogen liberation is higher in calculus-forming groups, thus tending to keep the calculus deposits growing. The results of this study may give a clearer understanding of the original experiment of Prinz[5] who tried to show that as saliva settles out and has a chance to stagnate, there develops more ammonia and, subsequently, calculus forms. It would also substantiate the findings of Kirk, who claimed that an increased ammonia concentration is the cause of calculus deposition.

EXPERIMENTAL PROCEDURES

Saliva samples from 102 patients, who varied from 25 to 60 years of age, were obtained from the Dental Admitting Clinic of the University of Illinois, College of Dentistry. All the samples tested were obtained between the hours of 10 and 12 in the morning or 2 and 4 in the afternoon. In either case it was at least 2 hours after the last meal. The patients were classified into the following groups: Calculus-Free, Calculus-Present, and Periodontal-Diseased. Roentgenologic and mouth examinations were preformed to determine the classification of the patient. Salivary *Lactobacillus acidophilus* counts were also made to help in the classification of the patient. In this study 1,020 ammonia nitrogen determinations were preformed.

The Calculus-Present Group was further subdivided into the following:

A. *Slight formers* characterized by small amounts of calculus deposits on any of the teeth. Usually the interproximal and lingual surfaces of the lower incisors and the buccal surfaces of the upper molars had a slight deposit.

B. *Moderate formers* characterized by a larger amount of calculus covering most of the lingual surfaces of the lower anterior teeth coming to a thickness of the width of the lingual marginal gingiva. The upper molar teeth were also usually involved as well as the other teeth.

C. *Abundant formers* characterized by an excessive amount of calculus deposited on many of the teeth, covering the entire lingual and interproximal surfaces of the lower anterior teeth and the buccal surfaces of the upper molars. A considerable amount was also found on other teeth.

The Calculus-Free Group was subdivided into the folowing:

A. *Caries-negative*—no apparent unfilled lesions and no fillings inserted during previous year. The *L. acidophilus* counts were usually negative, or below 10,000 per c.c. saliva.

B. *Caries-moderate*—1 to 4 unfilled carious lesions or recent fillings. The *L. acidophilus* counts varied between 10,000 and 50,000 per c.c. saliva.

C. *Caries-abundant*—5 or more unfilled carious lesions or recent fillings. The *L. acidophilus* counts were greater than 50,000 per c.c. of saliva.

The Periodontal-Diseased Group was subdivided into the following:

A. *Gingivitis*—marginal gingiva inflammed with slight pockets in some cases.

B. *Periodontitis*—definite pockets, inflammation of the marginal gingiva, some alveolar bone destruction and loose teeth. Calculus was usually present.

C. *Periodontosis*—no inflammation, much alveolar bone destruction, teeth may be loose and migrated.

A 3-minute paraffin-stimulated sample of saliva was collected in a sterile container. This provided a stimulated sample for the *L. acidophilus* determination. After this at least 50 ml. of saliva were collected in another sterile container for the ammonia nitrogen determinations. These determinations were made on the day the saliva was collected and on the third, fifth, seventh, and ninth subsequent day. This interval was found best from initial experimental trials, since the peak of ammonia nitrogen content was usually within this period. One determination was made after the peak was reached.

Immediately after the samples were collected, the following procedures were performed on all the samples. The 3-minute stimulated sample was used for the *L. acidophilus* count following the technic of Hadley, Bunting, and Delves.[27] Ten milliliters of saliva were then pipetted into a 15 ml. conical volumetric centrifuge tube from the 50 ml. of collected saliva. Before the samples were removed the vessel was well shaken to insure a uniform distribution of the saliva. A sterile technic was employed throughout these experiments. The remaining 40 ml. was placed in a 37° C. incubator where it was to remain for 9 days, being removed only on the third, fifth, seventh, and ninth day for a 10 ml. sample which was tested according to the following procedure. The saliva was centrifuged at 4000 r.p.m. for 15 minutes. After this time 1 ml. of the supernatant liquid was removed and tested for its ammonia nitrogen content. The volume of the sediment was read and the remaining liquid was discarded and

the tube inverted and allowed to drain for 5 minutes. The remaining sediment was then resuspended in distilled water and the entire suspension, along with two subsequent washings, was tested for its ammonia nitrogen content. The amount of water used for the suspension and washings was approximately 10 ml. The ammonia nitrogen determination was then carried out using the Folin-Bell permutit method.[28] The Klett colorimeter was used for the colorimetric readings after the nesslerization process was completed. The results were calculated as to the number of milligrams of ammonia nitrogen in 100 ml. of the supernatant liquid or in the sediment remaining from 100 ml. centrifuged saliva.

The collection of the saliva took between 15 and 30 minutes. The color of the saliva was noted as well as the general turbidity. After chewing for this period of time it was found that the teeth became fairly clean, indicating that loose plaques, epithelial cells, and other debris all entered the saliva specimen. Also in those cases where there was gingival disease, blood was a common content of the sample, as well as the purulent exudate that is found in deep periodontal pockets. Therefore chewing for this length of time indicated the condition of the gingival tissues as to their ability to withstand normal masticatory function. Invariably the color of the saliva of those individuals with periodontal disease was of blood-tinged or a light brown color. Those with calculus sometimes had a similar picture depending upon the amount of calculus deposited on their teeth. The calculus-free group usually had a fairly clear, light-colored, frothy, opalescent saliva. It is of interest to note the amount and type of sediment of the three groups studied. The sediments were measured by reading the volume on the volumetric centrifuge tube after centrifugation. It was found to be fairly constant for the type of saliva tested. The periodontal-diseased group always had the heaviest sediment on the first day. All the sediments decreased in volume as the incubation period progressed. This was most likely being due to the breakdown of some of the material to soluble or colloidal products that were not affected by centrifugation.

The salivary specimen bottles were tightly stoppered at all times in the incubator. Hall and Westbay[29] have shown that by keeping containers stoppered more constant results are obtained in guarding against ammonia loss.

In counting the *L. acidophilus*, it was noted that where the ammonia nitrogen was very high, the bacterial count was correspondingly low. However, in a few cases there were large numbers of streptococci on the plate. This interesting fact indicates that the higher ammonia nitrogen readings may be associated with a high streptococci count. In 1921, Ayers, Rupp, and Mudge[30] showed that there exists several classes of streptococci capable of producing ammonia from peptones. The proteolytic streptococci may be the one found here.

RESULTS

The experimental findings have been arranged in a series of four tables. Table I contains the averages of the ammonia nitrogen content of the various salivary samples used during the 9-day incubation period.

TABLE I

MEAN VALUES OF AMMONIA NITROGEN CONTENT OF SALIVA SAMPLES

(Values in mg. %)

GROUP	DAYS OF INCUBATION				
	0	3	5	7	9
Supernatant Liquid					
Calculus-present	7 ± 0.31*	37 ± 1.4	34 ± 1.7	31 ± 1.9	23 ± 1.6
Calculus-free	7 ± 0.31	35 ± 1.2	35 ± 1.1	35 ± 1.9	28 ± 1.6
Periodontal-diseased	9 ± 0.66	44 ± 3.0	45 ± 3.7	38 ± 3.0	36 ± 3.5
Sediment					
Calculus-present	0.7 ± 0.03	1 ± 0.04	0.9 ± 0.03	0.9 ± 0.03	0.7 ± 0.03
Calculus-free	0.7 ± 0.04	1 ± 0.03	1.0 ± 0.04	1.0 ± 0.04	0.9 ± 0.04
Periodontal-diseased	0.9 ± 0.08	1 ± 0.08	1.0 ± 0.08	1.0 ± 0.08	1.0 ± 0.08

*± .31 represents the standard error of the mean. The greatest variation between the calculus-present and the calculus-free groups occurred on the third and the ninth day. The minimum difference occurred on the third day while the maximum difference occurred on the ninth day. For the third day the standard error of difference was:

$$\sigma D = \sqrt{(\sigma M_1)^2 + (\sigma M_2)^2} \quad \text{or} \quad \sqrt{(1.4)^2 + (1.2)^2} = 1.8 \quad {}^{2}/_{1.8} = 1.1$$

Since the difference of the means is only 1.1 times its standard error such a difference may be considered as without significance. For the ninth day the standard error of difference was:

$$\sqrt{(1.6)^2 + (1.7)^2} = 2.34 \quad {}^{5}/_{2.34} = 2.14$$

Since the difference of the means is slightly greater than two times its standard error, such a difference may be considered signficant.

Forty patients were placed in the calculus-present group. Of these 13 were slight calculus formers, 25 moderate calculus formers, and 2 abundant calculus formers. The number of unfilled, open carious lesions found with the aid of the mirror, explorer, and a complete set of roentgenograms was counted. It was observed that the majority in each group had few or no lesions. This was also substantiated by a salivary *L. acidophilus* determination. Table II summarizes this finding.

TABLE II

SUMMARY OF CALCULUS-PRESENT GROUP SUBDIVIDED ACCORDING TO THE CARIOUS LESIONS

AMOUNT OF CALCULUS DEPOSITED	NUMBER OF CARIOUS LESIONS FOUND		
	NONE (PER CENT)	1 TO 4 (PER CENT)	5 OR MORE (PER CENT)
Slight (13 cases)	61.5	15.3	23.2
Moderate (25 cases)	64.0	20.0	16.0
Abundant (2 cases)	100.0	0.0	0.0

The calculus-free group also contained 40 patients. Of these 13 had no unfilled carious lesions, 19 had 1 to 4 unfilled carious lesions, and 8 had 5 or more unfilled carious lesions. In about 25 per cent of these individuals the *L. acidophilus* count did not correlate with the clinical picture. However, in those cases where there were apparent unfilled carious lesions with a low *L. acidophilus* count, the lesions may have been arrested.

Twenty-two patients were placed in the periodontal-diseased group. Of these, 3 had gingivitis, 8 had periodontitis and 11 had periodontosis. The majority in this group had no unfilled carious lesions and a negative or very low *L. acidophilus* count. In 2 cases, a very high count was obtained. It was of interest to note that both of these patients wore partial dentures. Bradel and Blaney* have shown that individuals wearing partial dentures have an

*Personal communication with one of the authors (Blaney).

increased salivary lactobacillus count. The periodontal-diseased groups contained the greatest amount of sediment in their saliva. Table IV shows these findings.

TABLE III

AMMONIA NITROGEN CONTENT OF CALCULUS-FREE GROUP SUBDIVIDED ACCORDING TO THE NUMBER OF CARIOUS LESIONS

(Values in mg. %)

CARIOUS LESIONS	DAYS OF INCUBATION				
	0	3	5	7	9
Supernatant Liquid					
None (32.5% of cases)	6.6	36.2	33.7	33.2	31.7
1 to 4 (47.5% of cases)	7.6	33.4	34.5	31.1	27.5
5 or more (20.0% of cases)	7.1	36.7	38.2	33.5	25.0
Sediment					
None	0.71	1.10	0.94	0.90	0.84
1 to 4	0.73	0.99	1.00	0.97	0.91
5 or more	0.66	0.96	0.94	1.00	0.89

DISCUSSION

The separation of the sediment from the supernatant liquid by centrifugation can be considered a quick settling-out process. This may be analogous to what occurs in the mouth when saliva has an opportunity to remain there for a prolonged period of time. The incubation of the saliva can represent the stagnation that saliva undergoes in the mouth. It is obvious that the length of time used in this experiment is much greater than could occur in the mouth, but it does show what potential ammonia nitrogen results when saliva is allowed to stagnate. Most probably the nitrogenous material in saliva is broken down to smaller units such as the conversion of urea to ammonia, amino acids to ammonia and other products, and even the breakdown of mucin and other proteins to smaller units. Bacterial action may be necessary for these conversion processes. When samples of saliva were autoclaved and then tested for its ammonia nitrogen content, it was found that little ammonia nitrogen was produced. No growth occurred when the autoclaved samples were plated out on media having a pH of 5 and 7.

TABLE IV

CENTRIFUGED VOLUME OF THE SEDIMENTS

(Values in c.c./100 ml. saliva)

GROUP	DAYS OF INCUBATION				
	0	3	5	7	9
Calculus-free	1-3	1-2	0.5-2	0.5-1	0.5-1
Calculus-present	2-4	1-3	1.0-3	1.0-2	0.5-1
Periodontal-diseased	3-5	2-3	1.0-3	1.0-2	1.0-2

In summarizing the individual averages of the three classes of the calculus-free group (Table III), it was found that the class with no unfilled carious lesions was somewhat similar to the calculus present group for the first 5 days. This points to the possibility of a relationship between few or no carious lesions and the presence of calculus. This would then corroborate the findings of

Dreizen and Spies[31] who showed that there may be an antithetical relationship between caries and periodontal disease. Broderick[32] also stressed this point of antagonism and claimed it to be due to a general acidosis as in caries or a general alkalosis as in the periodontal diseases. It was postulated by Dreizen and Spies[31] that the products of protein putrefaction may prevent caries formation. Berg, Burrill, and Fosdick[33, 34] working with stagnating saliva, isolated some of these products and contemplated their usage for caries prevention. This experiment does not bring out every aspect of this controversy, but it does show that the amount of ammonia nitrogen capable of being formed in both groups is somewhat similar. Kesel, O'Donnell, Kirch, and Wach[35] have shown that a concentration of 40 to 50 mg. per cent of ammonia nitrogen is necessary to inhibit *L. acidophilus* growth. The findings of this experiment show that such a concentration is more frequently found in the saliva of individuals with periodontal disease than in those free of periodontal disease. From these and other clinical observations it was seen that periodontal-diseased individuals have the least amount of active caries and the lowest *L. acidophilus* count. These findings would then substantiate the fact that a low salivary *L. acidophilus* count is associated with a reduced caries activity.

CONCLUSIONS AND SUMMARY

1. The sediment and the supernatant liquid of centrifuged saliva from 40 calculus-present, 40 calculus-free, and 22 periodontal-diseased individuals were tested by over 1,000 determinations for their ammonia nitrogen content.

2. The samples were analyzed on the day they were obtained and on the third, fifth, seventh, and ninth day of incubation at 37° Centrigrade.

3. The supernatant liquids contained much more ammonia nitrogen than the sediments.

4. The saliva of the periodontal-diseased group contained the most ammonia nitrogen both in the supernatant liquid and in the sediment of all the groups tested.

5. A very significant difference of the total ammonia nitrogen in the supernatant liquid was noted between the periodontal-diseased group and the other two groups. There was no significant difference between the over-all ammonia nitrogen production of the calculus-present and the calculus-free groups during the first 7 days of incubation. However, between the seventh and ninth day of incubation the calculus-present group lost its ammonia nitrogen much more rapidly than the calculus-free group. Statistically, this loss was found to be significant.

6. The class with no carious lesions in the calculus-free group resembled the calculus-present group in the ammonia nitrogen content for the first 5 days of incubation.

7. The calculus-present group showed the majority to have few or no carious lesions. From the data obtained it may be surmised that an agency which tends to predispose to calculus formations also tends to inhibit, to some extent, carious lesions.

8. Bacterial action was found to be necessary for the formation of ammonia nitrogen in incubated saliva. However, other factors may be present in saliva which contribute to ammonia nitrogen formation.

9. Invariably the periodontal-diseased group had calculus deposits on their teeth as well as the greatest ammonia nitrogen content. From this it may be assumed that ammonia nitrogen may have some correlation with calculus deposition in the oral cavity. Other conditions in the mouth of the periodontal-diseased individuals may also contribute factors in calculus deposition. Further investigations, however, must be carried out before this factor can be determined.

10. The periodontal-diseased group contained few or no *L. acidophilus* in their saliva. There were few unfilled carious lesions in these individuals. It has been shown in other investigations that a concentration of 40 to 50 mg. per cent of ammonia nitrogen can inhibit growth of *L. acidophilus*. The salivary ammonia nitrogen content of the periodontal-diseased group was 45 mg. per cent. This represented the greatest average of the group after 3 days of incubation. From this, one may postulate a relation between few or no carious lesions, a low *L. acidophilus* count, and a high ammonia nitrogen content.

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SALIVARY AMMONIA AND ITS CORRELATION TO DENTAL CALCULUS

II. Ammonia Nitrogen Development From the Washed Residue of Centrifuged Saliva Over a Seventy-two-Hour Incubation Period

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THE PRODUCTION of salivary ammonia has taken on new significance since it has been intimated that it may be one of the agents active in caries immunity.[1-6] The presence of salivary ammonia may also be an important factor in the formation of dental calculus. This was postulated years ago but, to the present time, no conclusive evidence has been presented to substantiate this fact.[7-10] It has been shown in Part I of the present study[13] that the content of ammonia nitrogen produced in the saliva over a nine-day incubation period was not significantly different for individuals with or without dental calculus. The sediment from centrifuged saliva was also included in that study. The ammonia nitrogen determinations were made on the third, fifth, seventh, and ninth day of incubation. In the present study the sediment was more carefully studied. This was accomplished by thoroughly washing the sediment with sterile normal saline. The sediment was allowed to remain in the saline for seventy-two hours during which time four ammonia nitrogen determinations were made, one every twenty-four hours. The results of such procedures could show if a difference does exist in the ammonia nitrogen producing activity of the saliva from calculus formers and nonformers.

EXPERIMENTAL

A group of sixty patients from the admitting clinic of the University of Illinois, College of Dentistry, volunteered to supply the saliva samples used for this study. This group was classified according to the amount of calculus that was found deposited upon their teeth. Thirty patients were classified as calculus formers and thirty as nonformers. The calculus-present group was further classified into slight, moderate, and abundant formers. The basis of the classification is the same as the one used in Part I of the study.[13] *Lactobacillus acidophilus* counts were made on each specimen of saliva. At least two counts were made from the same patient and an average taken. The number of carious lesions was also counted and recorded. A full mouth examination with the aid of roentgenograms was performed on each patient. The gingival condition was also noted and recorded.

Saliva samples were collected between the hours of 10 and 12 in the morning or 2 and 4 in the afternoon, in either case at least two hours after the last meal.

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Three-minute specimens (paraffin stimulated) served as samples for the *L. acidophilus* counts and also as a means of removing food and other debris from the mouth. Another 15 c.c. sample was collected in a sterile container which was used for obtaining the sediment by centrifugation. The collecting vessels were stored in a 37° C. incubator when not in use. During the collection of the saliva the container was held tightly in the patient's hand so as to insure a temperature as close to body temperature as possible.

TABLE I

AMMONIA NITROGEN FROM SEDIMENT OF CENTRIFUGED SALIVA OF CALCULUS-FREE INDIVIDUALS
(Values in mg. per c.c. saliva)

PATIENT NO.	SEX	AGE (YRS.)	LESIONS	L. ACIDOPHILUS (AVG.)	PER CENT RESIDUE	0 HRS.	24 HRS.	48 HRS.	72 HRS.
1.	M	28	3	2,000	.65	0	.010	.045	.050
2.	M	27	4	191,000	.68	0	.012	.035	.050
3.	F	30	0	15,000	.70	0	.033	.102	.120
4.	M	27	0	19,000	.69	0	.025	.060	.074
5.	M	20	3	3,500	.75	0	.014	.026	.042
6.	F	28	rampant	10,000	.95	0	trace	.010	.012
7.	M	29	0	no growth	.79	0	.032	.095	.172
8.	F	25	0	350	.54	0	.010	.010	.012
9.	M	23	0	no growth	.88	0	.018	.036	.085
10.	M	25	0	1,500	.91	0	.024	.056	.100
11.	M	25	0	191,000	.63	0	.010	.015	.002
12.	F	31	5	500	.53	0	.014	.025	.001
13.	F	35	3	1,500	.65	0	.022	.140	.030
14.	M	27	0	no growth	.84	0	.042	.125	.180
15.	F	30	0	5,000	.85	0	.015	.040	.070
16.	M	27	4	131,000	.64	0	.018	.015	.028
17.	M	28	0	30,000	.91	0	.014	.048	.065
18.	F	41	0	no growth	.74	0	trace	.008	.032
19.	M	30	1	20,000	.59	0	.008	.040	.030
20.	F	36	0	no growth	.60	0	.012	.020	.014
21.	F	25	rampant	13,000	.43	0	.012	.025	.015
22.	M	29	0	106,000	.52	0	.014	.038	.025
23.	M	27	1	no growth	.55	0	.010	.015	.018
24.	M	33	0	no growth	.54	0	.015	.015	.032
25.	M	23	5	7,000	.53	0	trace	.010	.010
26.	F	25	0	no growth	.62	0	.010	.015	.012
27.	M	27	4	64,000	.63	0	.032	.032	.066
28.	M	24	0	no growth	.91	0	.066	.098	.160
29.	F	19	0	no growth	.54	0	.008	.025	.012
30.	F	28	0	15,000	.59	0	trace	.018	.008
Over-all average					.68		.017	.038	.051

After collection the following procedures were performed on all the samples: Ten c.c. of saliva were pipetted into a sterile five-inch test tube, stoppered with a sterile cotton plug, and allowed to centrifuge for fifteen minutes at 4000 r.p.m. During this time a 1 c.c. sample was pipetted into a constant weight, small white porcelain crucible and weighed with a chain type analytical balance. The crucible was then placed in a drying oven where it was to remain for twenty-four hours and again weighed until a constant value was obtained. The percentage residue was then calculated. The sample that was collected for the *L. acidophilus* count was now used. The determination was performed according to the method of Hadley, Bunting, and Delves.[11]

After the centrifugation process was completed, the supernatant liquid was discarded and 10 c.c. of sterile saline added to the tube containing the sediment. The sediment was resuspended with the aid of a sterile bacteriological loop and again centrifuged for five minutes. This was continued until the remaining supernatant liquid was crystal clear. Usually four to six centrifugations were necessary. When the final centrifugation was completed a 1 c.c. sample was removed and an ammonia nitrogen determination performed. The sediment

TABLE II
AMMONIA NITROGEN FROM SEDIMENT OF CENTRIFUGED SALIVA OF CALCULUS-PRESENT INDIVIDUALS
(Values in mg. per c.c. saliva)

PATIENT NO.	SEX	AGE (YRS.)	CALCULUS*	LESIONS	L. ACIDOPHILUS (AVG.)	PER CENT RESIDUE	0 HRS.	24 HRS.	48 HRS.	72 HRS.
1.	F	31	S	2	250	.45	0	trace	trace	.012
2.	F	21	P-A	0	no growth	.72	0	.044	.165	.232
3.	F	26	M	6	no growth	.46	0	.010	.002	.025
4.	F	40	M	3	no growth	.71	0	.004	.012	.028
5.	F	30	P-S	2	no growth	.67	0	.012	.025	.070
6.	M	25	G-S	0	no growth	.63	0	.002	.008	.018
7.	M	25	P-M	0	no growth	.72	0	.025	.055	.040
8.	M	40	G-M	0	no growth	.90	0	.050	.105	.135
9.	M	25	S	0	132,000	.82	0	.014	.018	.020
10.	F	30	S	1	no growth	.68	0	.010	.015	.040
11.	F	46	P-M	0	346,000	.75	0	.015	.018	.040
12.	F	26	M	6	315,000	.63	0	.024	.050	.054
13.	F	40	S	1	4,500	.76	0	trace	.010	trace
14.	F	60	A	4	252,000	.67	0	.038	.044	.080
15.	F	21	G-S	4	no growth	.55	0	.015	.018	.018
16.	F	29	S	2	300	.73	0	.015	.015	.005
17.	F	20	S	3	31,000	.55	0	trace	.012	.012
18.	F	40	S	0	no growth	.63	0	.030	.020	.026
19.	F	35	P-M	0	no growth	.88	0	.028	.024	.012
20.	F	28	S	0	no growth	.55	0	.080	.095	.102
21.	F	37	M	2	3,000	.72	0	.014	.028	.038
22.	F	29	S	rampant	10,000	.56	0	.032	.015	.022
23.	F	44	P-M	5	2,000	.67	0	.034	.044	.080
24.	F	40	M	1	7,500	.59	0	.014	.022	.048
25.	F	10	M	0	300	.71	0	.022	.040	.032
26.	M	34	P-S	1	195,000	.54	0	.015	.016	.014
27.	F	25	G-S	0	no growth	.64	0	.008	.010	.005
28.	M	54	M	0	no growth	.64	0	.012	.018	.022
29.	M	42	A	0	no growth	.83	0	.025	.040	.060
30.	F	34	S	2	no growth	.63	0	trace	.010	trace
Over-all average						.66		.020	.032	.043

*Classification of patients:
S = Slight calculus deposits
M = Moderate calculus deposits
A = Abundant calculus deposits
G = Gingivitis
P = Periodontitis

was again resuspended, stoppered with the cotton plug, and placed in the 37° C. incubator. Every twenty-four hours the tube was removed from the incubator and again centrifuged for five minutes. Again another 1 c.c. sample was removed and tested for its ammonia nitrogen content. This process was repeated four times or a total incubation period of seventy-two hours. The results were tabulated in two tables and a statistical evaluation was made. (Tables I and II.)

A modified Folin-Bell permutit method was used for the determination of the ammonia nitrogen. The modification consisted in using the Coleman (model 14) spectrophotometer for the color comparisons instead of the visual method. This machine is very sensative for concentrations of ammonia nitrogen between 0 and 0.50 mg. per c.c. The readings were taken at 410 millimicrons employing a pc-4 filter. The reagent blank was always set at 100 per cent transmission.[12]

SUMMARY AND CONCLUSIONS

Statistically, no difference could be found between the ammonia nitrogen production of the washed salivary sediment from calculus-free and calculus-present individuals. The calculus-present group showed, on the average, lower salivary lactobacillus counts. The residue calculated in percentage of the total sample weight revealed the following results: calculus-free residue, 0.68 ± 0.025; calculus-present residue, 0.66 ± 0.019. Statistical analysis of these results again indicated no significant difference between the two groups. (Table III.)

TABLE III
STATISTICAL EVALUATION

PER CENT RESIDUE	INCUBATION TIME 24 hrs.	48 hrs.	72 hrs.
I. Calculus-Free Individuals			
Mean	.68	.017	.038	.051
Standard Deviation*	.14	.0135	.0298	.0496
Standard error of mean†	.025	.0024	.0054	.0089
II. Calculus-Present Individuals			
Mean	.66	.020	.032	.043
Standard Deviation	.109	.0168	.0243	.0463
Standard error of mean	.019	.0030	.0044	.0084
III. Statistical Comparison of Calculus-Free and Calculus-Present Group			
"t" value‡	.64	.79	.87	.65
Probability§	.50	.50	.40	.50
Significance as to correlation	None	None	None	None

*Standard deviation $\sigma = \sqrt{\Sigma d^2/N}$

†Standard error of mean $\sigma_M = \sigma/\sqrt{N}$

‡"t" value or the standard error of difference is: $\frac{X^1 - X^2}{\sqrt{(\sigma_{M1})^2 + (\sigma_{M2})^2}}$

$X_1 - X_2$ = actual difference between means.

§Probability values taken from Fisher "t" table of statistical significance.

The production of ammonia nitrogen in many of the past studies was shown to be due to the enzymatic conversion of amino acids and urea. Washing of the sediment in this experiment removed these soluble components. Yet it was shown that ammonia nitrogen could be produced from the washed salivary sediment. One could postulate that this ammonia nitrogen production may have been due to proteolytic decomposition of certain elements in the sediment by bacterial and or cellular enzymes. Amongst these elements are mucin, epithelial cells, bacteria, and debris. Each of these could be studied in relation to the ammonia nitrogen development in saliva.

The patients with gingival disease in this study did not show a greater weight of the per cent residue over that of the normal cases. In the previous

paper it was shown that the volume of the sediment resulting from centrifugation was slightly more in those cases where gingival disease was evident.

It would appear, therefore, from this present study as in the earlier study,[13] that there is little, if any, relationship between the ammonia nitrogen content and production ability of the saliva from calculus formers compared to non-formers. However, the factor of ammonia and calculus formation cannot as yet be ruled out. Of prime importance for future studies is a more objective criteria for calculus classification as well as a more careful study of individuals who are susceptible to calculus formations and present no caries or other disease to prevent a clear picture.

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SALIVARY AMMONIA AND ITS CORRELATION TO DENTAL CALCULUS

III. The Fate of Salivary Mucin, Nonmucin, and Ammonia Nitrogen Over a Seventy-two-Hour Incubation Period

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THE SOURCE of ammonia nitrogen in human saliva has been shown to be amino acids and urea.[1] The presence of deaminating systems in saliva have also been proved.[2, 3] When saliva is permitted to stagnate, it undergoes enzymatic changes and breaks down to smaller units with additional formation of ammonia nitrogen. Individuals with periodontal disturbances have a tendency to show more rapid salivary breakdown than do normal individuals.[4, 5, 6] The time limit of saliva remaining in the oral cavity has never been calculated with any degree of accuracy. It may remain in the mouth from a few minutes to many hours depending upon its location within the oral cavity and also the degree of activity of the individual. If saliva has an opportunity to remain in the oral cavity for more than a few minutes, as it certainly does when an individual is asleep, many enzymatic reactions have time to occur. This was one of the hypothesis of the author when the study which is the basis of this paper was undertaken.

It is assumed by many investigators that the amino acids and urea that are found in human saliva are filtered into the oral cavity through the salivary glands. The present study was undertaken to determine if ammonia nitrogen could be formed by the breakdown of mucin and nonmucin compounds and the rate at which this might occur in the various groups studied. The groups studied were calculus-present, calculus-free, and periodontal-diseased individuals. It would also be of interest to ascertain if the rate of breakdown of the mucin and nonmucin substances varies with the different groups studied.

EXPERIMENTAL

Saliva samples from thirty-three staff members and students from the University of Illinois, College of Dentistry, were used in this experiment. This group was classified according to the amount of calculus deposits and gingival disease. Three groups were formed: calculus-present, calculus-free, and periodontal-diseased. Full mouth examinations with the aid of a complete set of roentgenograms were performed. The basis of this classification can be found in Part I of the present study.[9] Eleven individuals were placed in each of the three groups.

Saliva samples were collected usually two hours or more after the morning meal. Approximately a three-minute specimen (paraffin stimulated) served as

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a vehicle for removing excessive remaining food debris. This sample was discarded. The next 35 ml. of saliva was collected in a sterile container, which was tightly held in the patient's hand to insure that the specimen be as close to body temperature as possible. These containers previously had been stored in a 37° C. incubator. Immediately after collecting, and every twenty-four hours thereafter (for a total period of seventy-two hours) the following procedures were performed:

The total nitrogen was determined by the technic of Koch and McMeekin.[7] One c.c. of a 1:3 diluted sample of saliva was used for testing. The saliva was always well shaken before removing a sample from the collecting container. The results were calculated in milligrams per cent of nitrogen.

The nonmucin nitrogen determinations were made with the technic of Youngberg.[8] This method involves the precipitation of the mucin by employing a 1 per cent trichloroacetic acid solution. The ratio used was 5.0 c.c. of saliva to 7.0 c.c. of the trichloroacetic acid. After adding the saliva to the acid in a five-inch test tube it was allowed to centrifuge for five minutes at 4,000 r.p.m. This procedure brought the precipitated mucin to the bottom of the tube fairly well packed. Of the supernatant liquid (this volume containing 1.0 c.c. of saliva) 2.4 c.c. were used for the nonmucin nitrogen determination. The results were calculated in mg. per cent of nonmucin nitrogen. After these results were obtained they were subtracted from the total nitrogen. This gave the value of the mucin nitrogen.

The ammonia nitrogen determinations were secured by the Folin-Bell permutit method, with one modification. This modification consisted of using the Coleman (model 14) spectrophotometer instead of the usual visual method for the colorimetric readings. The wave length was set at 410 millimicrons. A pc-4 filter was also employed. The blanks were always set for 100 per cent transmittance. The results were calculated in mg. per cent of ammonia nitrogen; 2.4 c.c. of the supernatant liquid obtained from the nonmucin nitrogen determination was also used for the ammonia nitrogen determination.

CONCLUSIONS AND SUMMARY

As the saliva stagnated the mucin nitrogen of all groups declined. The greatest decrease occurred during the first twenty-four hours in all the groups. There was a small decline in the mucin nitrogen for the periodontal-diseased group between the twenty-fourth and seventy-second hour of incubation. The calculus-present and the calculus-free groups showed a small decline between the twenty-fourth and forty-eighth hour of incubation. Between the forty-eighth and seventy-second hour there was almost no change. (Tables I and II.)

The periodontal-diseased group contained the greatest concentration of nonmucin nitrogen initially and produced the most during the incubation period. The calculus-present group contained more nonmucin nitrogen than the calculus-free group from the beginning to the end of the incubation period. The graph shows the calculus-present and the calculus-free groups to be nearly parallel to

TABLE I
CALCULUS-PRESENT GROUP
(Values in mg. per cent)

CASE	AMT. CALCULUS	MUCIN NITROGEN				NONMUCIN NITROGEN				AMMONIA NITROGEN			
		0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.
1.	Slight	17.2	5.6	4.6	5.0	46.8	49.4	50.4	28.8	6.0	34.8	40.8	28.0
2.	Slight	19.6	14.0	10.1	9.0	25.4	30.0	31.2	11.5	10.0	18.2	22.1	11.0
3.	Moderate	17.8	9.3	5.7	6.9	37.2	42.0	45.6	33.6	6.5	33.6	34.8	21.1
4.	Slight	14.9	8.4	5.9	7.0	46.6	57.6	52.3	50.9	7.5	36.0	37.2	35.0
5.	Slight	21.0	15.6	9.8	10.6	42.0	47.0	44.2	40.4	3.5	28.8	25.0	14.4
6.	Moderate	16.2	9.0	8.8	12.6	34.8	36.0	35.0	34.8	1.5	24.0	21.6	25.2
7.	Moderate	17.8	15.0	11.4	10.8	56.6	60.0	57.6	58.8	3.6	39.6	43.2	45.6
8.	Moderate	17.2	12.6	11.5	9.8	37.4	40.8	36.5	39.4	9.0	36.0	28.8	30.0
9.	Moderate	13.2	12.0	10.8	8.5	40.8	48.0	43.2	45.5	7.0	37.2	40.8	42.5
10.	Slight	18.0	13.8	11.1	11.9	35.5	43.2	38.4	37.9	4.5	31.2	34.1	26.4
11.	Moderate	18.0	12.6	9.6	11.9	38.4	44.4	42.0	35.5	10.4	36.0	26.4	19.2
Over-all mean		17.3	11.6	9.0	9.4	40.1	45.3	43.3	37.9	6.3	32.3	32.2	27.1

TABLE II
CALCULUS-FREE GROUP
(Values in mg. per cent)

CASE	MUCIN NITROGEN				NONMUCIN NITROGEN				AMMONIA NITROGEN			
	0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.
1.	20.1	8.0	6.4	5.5	25.9	34.8	33.6	13.9	5.5	25.9	28.3	13.0
2.	17.4	10.9	6.5	4.9	26.4	32.6	37.0	34.1	4.5	24.0	32.4	24.0
3.	24.2	9.1	8.2	6.0	41.8	49.4	44.6	48.0	1.5	38.4	36.0	37.2
4.	19.2	9.3	8.7	7.8	40.8	55.2	52.8	52.8	4.0	44.4	45.6	46.8
5.	23.3	12.6	9.6	8.6	30.2	32.4	34.8	31.2	1.2	22.8	22.8	19.2
6.	14.3	6.4	7.8	5.5	33.1	39.8	38.4	36.5	6.0	28.8	29.3	26.4
7.	21.0	13.8	11.5	13.8	28.8	34.8	34.1	31.2	5.8	19.7	24.0	26.4
8.	13.8	13.1	15.2	12.0	33.6	37.9	32.2	24.0	8.0	21.1	18.7	12.0
9.	14.3	7.1	5.0	3.5	33.1	37.9	33.6	30.5	5.2	22.8	22.6	21.3
10.	14.7	17.1	19.5	16.2	52.8	50.4	42.0	40.8	2.9	22.1	30.7	34.1
11.	16.8	7.9	9.0	13.8	46.8	55.7	54.0	57.6	1.2	40.8	48.0	50.9
Mean	18.1	10.4	9.7	8.8	35.7	41.9	39.7	36.4	4.1	28.2	30.7	28.3

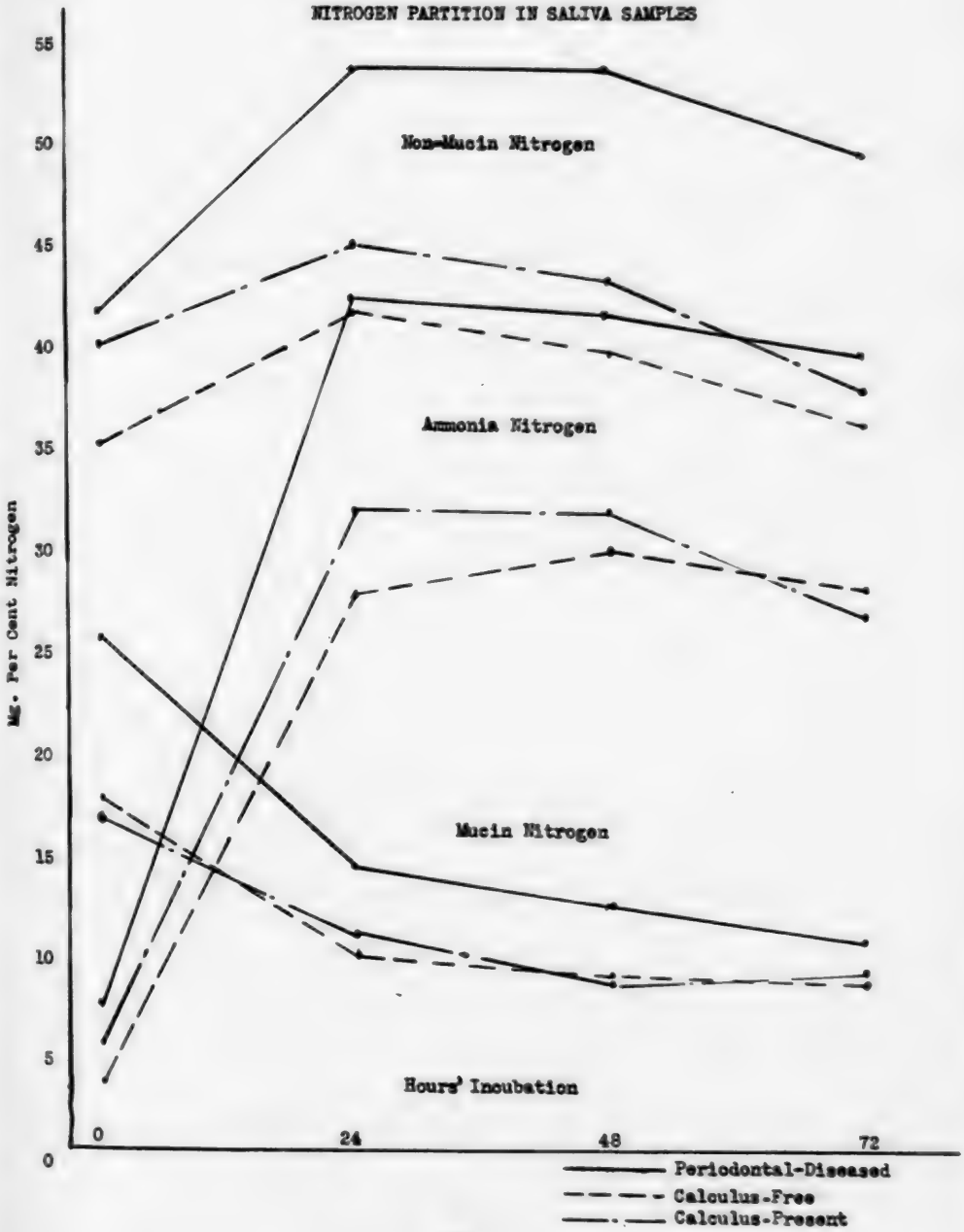
NITROGEN PARTITION IN SALIVA SAMPLES
Non-Mucin Nitrogen
Ammonia Nitrogen
Mucin Nitrogen
Mg. Per Cent Nitrogen
Hours' Incubation
0
5
10
15
20
25
30
35
40
45
50
55
0
24
48
72
Periodontal-Diseased
Calculus-Free
Calculus-Present

Fig. 1.

TABLE III
PERIODONTAL-DISEASED GROUP
(Values in mg. per cent)

CASE	PATHOLOGY*	MUCIN NITROGEN				NONMUCIN NITROGEN				AMMONIA NITROGEN			
		0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.	0 HRS.	24 HRS.	48 HRS.	72 HRS.
1.	P.A.	29.4	16.3	14.3	7.0	51.6	61.2	55.7	36.0	12.2	46.8	45.6	36.0
2.	P	22.8	9.8	5.3	6.6	37.2	43.2	49.2	38.9	6.2	37.2	32.4	29.8
3.	G	25.8	12.2	9.5	8.7	43.2	49.0	52.3	46.8	1.5	34.1	37.2	31.2
4.	P	22.1	11.4	8.4	7.8	49.9	57.6	61.2	58.8	1.0	41.3	45.6	55.2
5.	Po	31.2	21.6	20.1	14.4	52.8	57.6	56.4	55.2	6.0	43.7	43.7	39.6
6.	P.A.	20.2	8.4	11.4	7.1	29.3	39.6	33.6	37.9	9.0	37.2	29.8	31.2
7.	P	42.0	10.8	9.6	7.5	42.0	49.2	46.8	43.5	5.5	38.4	40.8	41.5
8.	P.A.	25.1	21.8	19.2	19.0	33.1	41.8	40.8	39.8	2.5	29.3	33.6	26.4
9.	Po	19.3	17.1	12.6	13.8	77.3	74.4	80.4	79.2	26.3	73.4	73.2	72.0
10.	P	25.5	15.0	18.0	20.4	42.0	46.8	48.0	42.0	8.2	37.0	37.0	30.0
11.	P	24.0	20.5	15.1	11.7	60.0	72.0	65.3	67.2	8.0	57.6	47.0	48.5
Over-all mean		26.1	14.9	13.0	11.2	47.1	53.8	53.6	49.5	7.8	43.2	42.3	40.1

*P.A. = Periodontal Atrophy
P = Periodontitis
G = Gingivitis
Po = Periodontosis

each other. The striking parallelism of the graphs would indicate that the rate and the amounts of nonmucin nitrogen formation in calculus-present and calculus-free individuals is largely dependent upon the initial nonmucin nitrogen content. (Table III.)

The periodontal-diseased group contained the most ammonia nitrogen and produced the most during the incubation period. The calculus-present group produced more ammonia nitrogen than the calculus-free group. This is in close agreement with the nonmucin nitrogen picture. However, between the forty-eighth and seventy-second hour of incubation, the calculus-free group contained slightly more ammonia nitrogen than the calculus-present group.

From this experiment it can be shown that as saliva undergoes stagnation the mucin nitrogen decreases, as the nonmucin nitrogen, as well as the ammonia nitrogen, increases in concentration. The rate of breakdown is greatest in the periodontal-diseased individuals. The calculus-present group contained a greater content of nonmucin and ammonia nitrogen than did the calculus-free group. The saliva of the former group also has the ability to form these at a more rapid rate over a seventy-two hour incubation period. This factor may be correlated with the presence of calculus deposits in individuals susceptible to it. However, more investigation must be performed before this can be conclusively determined. Future studies should concentrate on the fate of the nonmucin nitrogen during the first twenty-four hours of incubation. This may give a clearer picture as to the actual rate of salivary breakdown and also a better understanding of the chemistry of saliva of calculus formers and nonformers.

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THE EFFECT OF PROPYLTHIOURACIL ON THE PERIODONTIUM OF THE HAMSTER

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IT HAS been well established that hypothyroidism results in retarded bone growth and dentin formation, and delayed eruption of teeth.[1, 2, 3] Clinical observations have been reported which assert that an increase in incidence and/or severity of periodontal disease is seen among hypothyroids.[4-10] In the case of the cretin, it could be postulated that periodontal disease might develop due to the retardation of bone growth and eruption, and consequent disruption and crowding of the dental arches. An additional predisposition to periodontal disease might conceivably be due to the low mental aptitude of these patients and resultant lack of proper oral hygienic measures.

In the adult hypothyroid, or myxedematous individual, these considerations would not appear to be as important, as far as the periodontium is concerned. However, the impression gained from the clinical observations mentioned persists, in spite of opinions to the contrary,[11] and there is a lack of experimental evidence on the question.

Propylthiouracil,† a relatively nontoxic derivative of thiourea, is a goitrogenic drug which induces thyroid gland hyperplasia and inhibits thyroxine formation.[12, 13, 14] It is used for the treatment of hyperthyroidism in humans. Both the hamster[15] and rat[16] are said to be susceptible to its action. In light of previous studies[17] it seemed desirable to test the effects of this drug on the periodontium of the hamster.

METHODS

In the major portion of this study, 40 hamsters, 53 days of age, were distributed by sex and littermates into 2 equal groups. They were maintained on laboratory chow and distilled water ad libitum, with whole carrots as a supplement one day per week. The experimental group received 0.05 per cent propylthiouracil in water for 72 days, and the dosage was then increased to 0.075 per cent for 114 more days. At this time (186 experimental days) 5 males and 5 females of each group were sacrificed. The remaining animals were maintained for an additional 263 days on 0.1 per cent propylthiouracil (449 experimental days).

At sacrifice, the jaws of all animals were examined by means of a dissecting microscope (magnifications up to ×30). The jaws of the 20 animals sacrificed after 186 experimental days were fixed in formalin and embedded in celloidin for sectioning. After measuring its length, a femur from each animal was

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†"Probacil" (6-n-propyl-2-mercapto-4 hydroxypyrimidine), generously furnished by Lederle Laboratories, Division of American Cyanamid Co., New York, N. Y.

treated similarly, and the thyroid glands of all were prepared in paraffin. Four animals representative of those sacrificed after 449 days were prepared for study in the same way.

In addition, several litters of animals raised under the continuous influence of the drug through second, third, and fourth generations were observed and studied histologically.

During the experimental period, water (and drug) consumption was measured and weight curves were maintained. Activity indices were prepared[18] and attempts were made to measure the oxygen consumption and blood serum cholesterol[19] of some of the experimental and control animals.

RESULTS

From the measurements of water consumption of the 2 groups, made at intervals over a period of a year, it was ascertained that the average intake for each adult animal was approximately 8 c.c./day. There was no significant difference in intake between the experimentals and controls. The approximate dosage of propylthiouracil received was as follows: for the 0.05 per cent period, 4 mg.; 0.075 per cent, 6 mg., and 0.1 per cent, 8 mg. per animal per day. On a weight basis, the dosage for the latter 2 periods was calculated at 60 and 80 mg./kg. hamster/day. In view of previous studies of rats[20, 21] and hamsters[15] made in connection with goitrogenic drugs, it was felt that this dosage was adequate.

In view of the findings, it is believed that a moderate degree of hypothyroidism was produced and maintained for long periods of time. There was a consistent lag in weight gain among the treated animals. The average end weight of the control animals was 121 grams, and that of the treated animals was 108.4 grams. This lag was particularly marked in the later generations. Fig. 1 represents the weight curves for a litter of control and a litter of experimental animals (which were conceived and raised under the influence of 0.1 per cent propylthiouracil). The increase in weight of the controls from weaning to 64 days of age is remarkable compared to the experimentals.

On the basis of femur length, bone growth was less in the treated animals than in their controls; average length of the former was 26.21 mm., and that of the latter, 27.26 mm. It may be seen that the discrepancy in femur length corresponds to the weight lag of the experimental animals; thus, it is believed that the weight difference was a function of growth, rather than of body fat or other factors. In addition, histologic sections of the distal end of the experimental and control femurs showed delay in bone growth, as evidenced by a less advanced development of bony trabeculations of the experimental metaphyses.

The thyroid glands of all the experimental animals were tremendously enlarged, presumably due to a decrease in thyroxine production and consequent increase in the thyrotropic hormone formation, which stimulated a "futile" overgrowth[21] (Fig. 2). The microscopic appearance of these tissues is illustrated in Fig. 3, normal, and Fig. 4, treated. Note the lack of uniformity in follicle shape and size, papillary hyperplasia and distribution of colloid in the experi-

mental gland. These findings are in agreement with those of previous investigators.[12, 15]

The data obtained from the activity studies, basal oxygen consumption, and serum cholesterol measurements were inconclusive.[22] There were no positive signs of toxicity.

As studied on a comparative basis, the jaws revealed no significant differences between the treated and control groups. No measurements were made of these structures. Presumably the jaws of the treated animals might have been smaller than their controls as evidenced by the femur studies. Such a finding would be in agreement with those of previous investigators.[23]

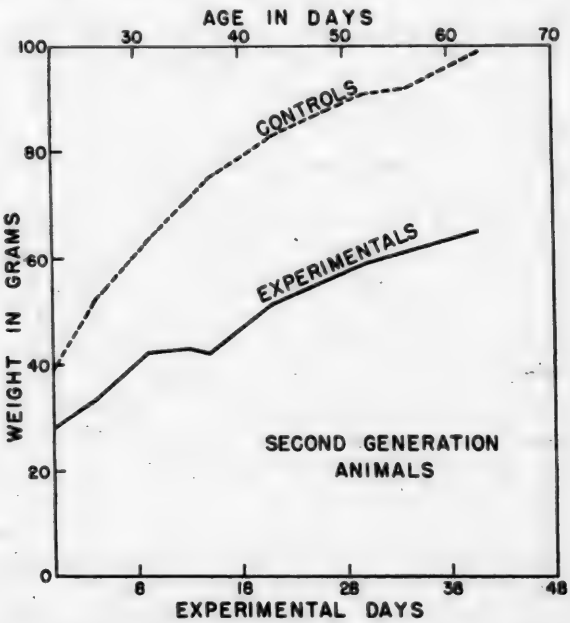
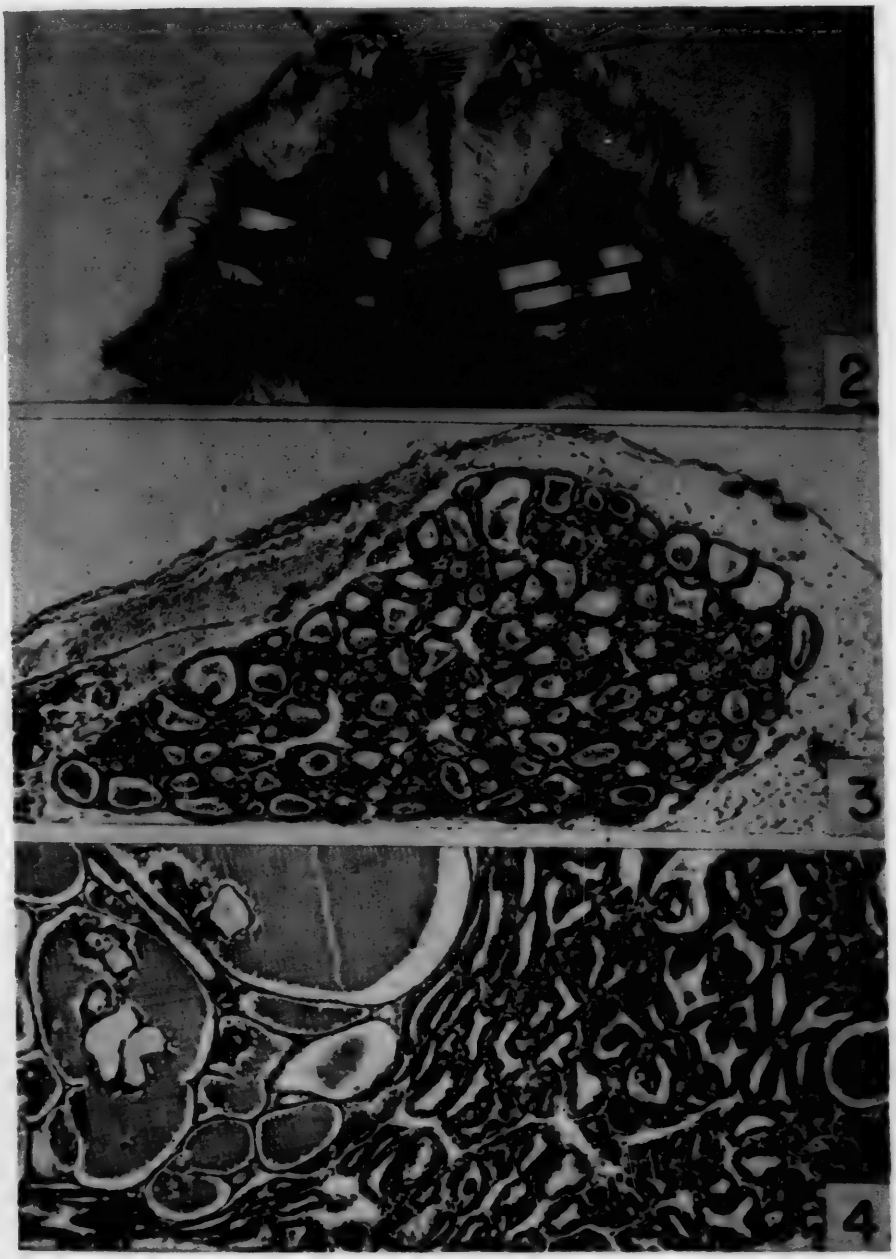


Fig. 1.—Weight curves of control and propylthiouracil-treated litter. Marked lag in weight gain of latter is apparent.

Practically no periodontal disease was noted grossly in those animals sacrificed after 186 days of the experiment; however, microscopic studies revealed that all of these animals had varying degrees of periodontal disease accompanied by small subgingival deposits. Fig. 5 shows a buccolingual section of the maxilla of a female, and illustrates the basophilic subgingival accumulations and early periodontal destruction found especially on the palatal surface. Other lesions associated with hair, food debris, and wood shavings (used for bedding) were common in both groups. The types of sections studied included buccolingual and mesiodistal directional cuts of maxillary and mandibular molars.

Of those animals sacrificed after 449 experimental days, generally speaking, all the forms of dental and periodontal disease encountered in the younger animals were present to a greater degree. Fifteen of these animals presented



Figs. 2 to 4.

Fig. 2.—Thyroid glandular hyperplasia in female treated with propylthiouracil for 186 days, and normal gland of female littermate control animal.

Fig. 3.—Section from normal thyroid gland of an animal 239 days of age.

Fig. 4.—Hyperplastic thyroid gland of treated littermate of the animal whose gland is shown in Fig. 3. Note congestion, cellular hyperplasia with papillary foldings into follicle lumina, and irregular distribution of colloid. (Same magnification as Fig. 3.)

gross periodontal disease, usually present in one or more localized areas, most commonly in the interproximal spaces. No single type of generalized periodontal destruction was seen. Gingival recession was found most frequently on the mesiolingual aspect of the lower first molars. Gingival hypertrophy, occasional carious lesions, and dental abrasion were encountered. A definite pattern of wear was observed, showing graded differences most extensive on the first, less on the second, and least on the third molars. The result resembled the compensating curve of wear seen in the human.

Those animals of the second and third generations which were born and raised under continuous exposure to propylthiouracil presented essentially the same histological findings as their controls. Grossly, they appeared to be dwarfs.

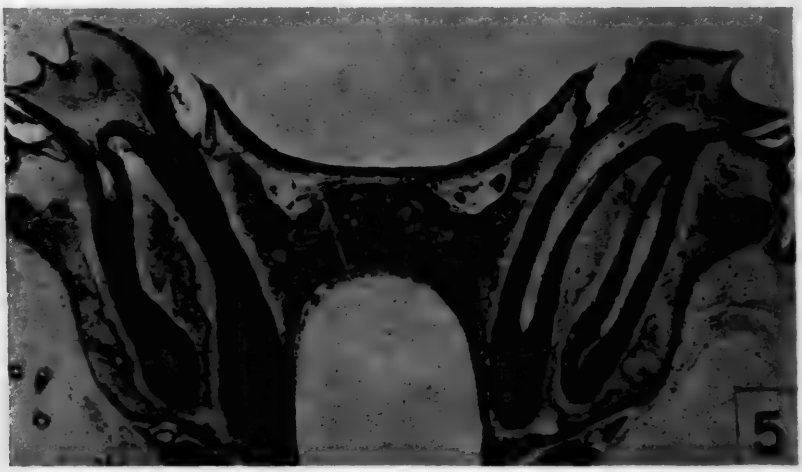


Fig. 5.—A buccolingual section of maxilla of treated female. Note subgingival accumulations and early periodontal destruction on palatal surfaces. Such findings were common to treated and control animals.

The fact that periodontal disease was so common among all the animals is indicative of the fact that the animals were susceptible to such attack, and that predisposing factors were present to further or insure the progress of any periodontal disturbance that might have been the direct result of the administration of propylthiouracil.

COMMENT

In spite of the inability to present incontrovertible proof that hypothyroidism was produced in these animals, there is considerable evidence that a prolonged antithyroid effect was present. The literature on the relationship between periodontal disease and hypothyroidism presents conflicting opinions. The negative findings of this study tend to reemphasize the doubt that there is any *direct* cause and effect relationship between these two conditions. One might not expect the rodent cretin (which the second, third, and fourth generation animals of this study resembled) to develop undue periodontal lesions on the basis of delayed dentition and crowding, since the arch form of the primates is not common to this order.

SUMMARY AND CONCLUSIONS

An effort has been made to determine the effects of hypothyroidism, as induced by the administration of propylthiouracil, on the periodontium of the hamster. Growth was inhibited in the experimental animals as evidenced by a lag in weight gain, decreased femur length, and histological findings in the femurs. The thyroid glands exhibited remarkable hyperplasia.

Gross and histologic studies revealed that periodontal disease was common in the experimental and control groups. Other functional and pathological processes concerned with the teeth and periodontium were observed.

As studied, there was no observable increase in periodontal pathology due to the administration of this drug. Supplemental studies of animals conceived and raised through four generations of continuous exposure to propylthiouracil corroborated these findings.

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HYALURONIDASE ACTIVITY IN HUMAN SALIVA

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THE HYALURONIDASES are a group of mucolytic enzymes[1, 2, 3] which hydrolyze hyaluronic acid, a mucopolysaccharide found in tissue of mesodermal origin—connective tissue and intercellular cement substance.[1, 4, 5, 6]

Many papers concerned with hyaluronidases have appeared in recent medical literature, where they are sometimes classified as "spreading factors," because they have the power to increase tissue permeability. Under certain conditions these factors enhance the spread of infection.[1, 7, 8, 9] Spreading factors have been demonstrated in several body fluids and testicular, pancreatic, skin, spleen, and salivary gland extracts.[4, 8-12]

Saliva has also been demonstrated to aid in the spread of infection.[13] A spreading factor, which was obtained from salivary gland extracts, has been named kallikrein. The spreading effect of this substance is known but its enzymatic action has not been demonstrated.[11, 12] Other possible sources of the spreading factors or hyaluronidase in saliva are cocci,[1, 14-18] which may be found in the dentinal tubuli, plaques, calcareous deposits, and gingival tissue affected by inflammatory disease.

If the hyaluronidase activity of saliva or oral tissues varies appreciably, this variation may provide a clue to the invasiveness of microorganisms that are universally present in the oral cavity. The hyaluronidase activity of saliva has not been demonstrated previously. The purpose of this investigation was to determine the presence of hyaluronidase activity and the variation from person to person.

EXPERIMENTAL PROCEDURE

Ten c.c. of saliva were obtained from each subject between 9:30 and 11:00 A.M. without paraffin stimulation. The saliva was collected in sterile centrifuge tubes, centrifuged for 45 minutes at 3500 r.p.m., and the supernatant liquid was dialyzed in cellophane bags against distilled water for at least 36 hours at 4° C. in order to remove salts. The technic selected for determination of hyaluronidase activity is that used by Hadidian and Pirie,[19] making use of the principle of viscosity reduction of a hyaluronic acid solution. The solution with unknown hyaluronidase activity acts upon a hyaluronic acid substrate producing varying rates of reduction in viscosity.

Hyaluronic acid substrate was prepared according to the pyridine method of Hadidian and Pirie.[19] Two c.c. of the substrate solution containing the proper amount of phosphate buffer and NaCl[19] were added to 2 c.c. of centrifuged and dialyzed saliva. The mixture was then transferred to an Ostwald viscosometric tube at 25° C. Measurements in viscosity were made every 2

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minutes during the first 10 minutes, every 5 minutes for the next 20 minutes, and every 10 minutes for the following 30 minutes. If little or no change occurred during the first hour, readings were continued for 5 hours at 20-minute intervals.

The initial viscosity was determined by combining 2 c.c. of hyaluronic acid substrate with 2 c.c. of distilled water and checks were made at 2- to 4-hour intervals on this viscosity in order to ascertain that the substrate itself did not change in viscosity.

Occasionally, a sample of saliva increased the initial viscosity somewhat when it was added to the substrate mixture. In all instances the rate of decrease in viscosity on addition of saliva was so small that the first reading could be taken as equivalent to the initial viscosity without introducing significant error.

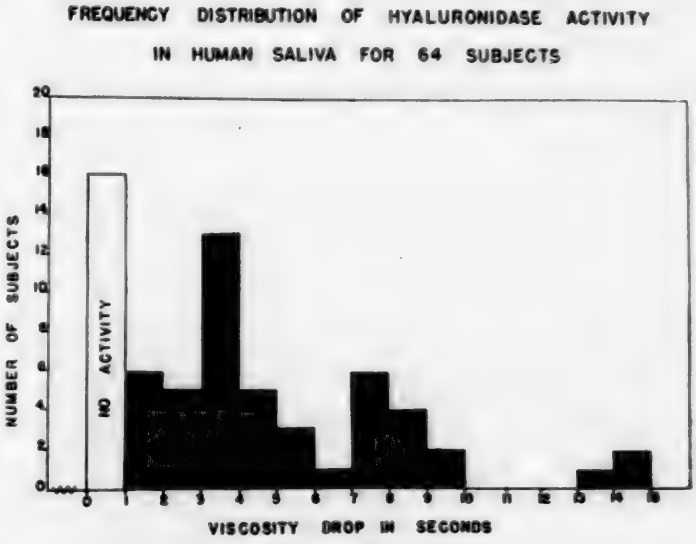
FREQUENCY DISTRIBUTION OF HYALURONIDASE ACTIVITY
IN HUMAN SALIVA FOR 64 SUBJECTS
NUMBER OF SUBJECTS
NO ACTIVITY
VISCOSITY DROP IN SECONDS

Fig. 1.

These tests were run initially on the saliva of 8 persons in order to learn whether hyaluronidase activity was usually present in saliva. Five of the samples showed a reduction of hyaluronic acid viscosity. Therefore, a more extensive investigation using the same method was undertaken with 64 individuals because this investigation might disclose its possible relationship to dental disease.

Sixty-four subjects were selected, ranging in age from 3 to 34 years. A clinical examination was made for all of these to determine the condition of their teeth and soft oral tissues.

Initial experiments showed that in the presence of saliva, there was a loss in the viscosity of hyaluronic acid. This loss occurred at a rate so slow that the use of half-time period, which is conventional in viscosometric determination

of hyaluronidase activity, could not be determined for practical reasons. Instead, it was decided to accept the change in viscosity during the first 45 minutes of the reactions as a measure of hyaluronidase activity. This activity is expressed as the difference in seconds between the flow time of the saliva-substrate mixture at zero time and at 45 minutes after the mixing of the substrate with saliva; in the figures this value is designated "viscosity drop."

RESULTS

The frequency distribution of hyaluronidase activity calculated in this manner is presented in Fig. 1 for the saliva of 64 subjects. Sixteen subjects had no activity. The distribution of hyaluronidase activity varies in the active group as indicated in Fig. 1. The frequency distribution of hyaluronidase activity is presented in Fig. 2 in a manner to show the median which appears at 3.4 seconds. Twenty-five per cent of the cases exhibit no hyaluronidase activity and another 25 per cent had activities greater than 7.5. The findings disclose that hyaluronidase activity is present in the saliva of 75 per cent of the persons examined and that among these, it ranges in relative activity from 1.0 to 14.5 seconds.

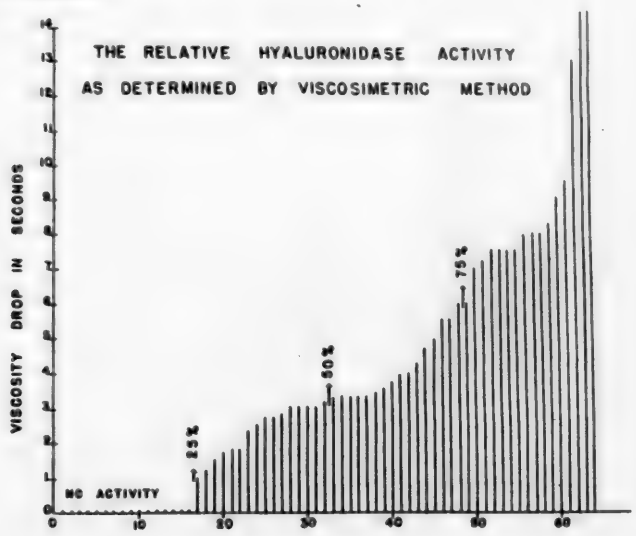

Fig. 2.

DISCUSSION

Twenty of the subjects presented some form of periodontal disease ranging from gingivitis to periodontoclasia. All of these had measurable hyaluronidase activity in the saliva. On the other hand, of the whole group, one-fourth of the subjects showed no activity in the saliva. No person in this group without hyaluronidase activity had any periodontal disease. Whether or not hyaluronidase has anything to do with periodontal disease must be determined by further investigation.

The numbers of decayed, missing, and filled teeth and the hyaluronidase activity of the saliva were correlated on 59 subjects. The correlation coefficient between missing and filled teeth and the hyaluronidase activity was − 0.013. The correlation coefficient between the enzyme activity and the DMF. was + 0.194. Neither of these coefficients was statistically significant. However, the correlation with numbers of carious teeth alone was + 0.442, and this was significant at the 1 per cent level.

Hyaluronidase is specific in its actions upon substances which contain hyaluronic acid or sulfuric acid esters.[1, 3, 16, 20, 21] These substances are present in the gingival tissues, the dental pulp, dentin, and blood vessels. Some bacteria have capsules composed of hyaluronic acid. The pH of saliva lies within the range at which the enzyme is known to be stable, thus it would be expected that enzyme action could take place in the oral cavity and surrounding tissues.[1] The hyaluronidase activity of saliva may be a factor in the initiation or progress of dental caries.

Hyaluronidase may aid in spreading caries of the dentin, where it might cause solution of the tubular contents or the organic portion of the matrix. Further investigations concerning this enzyme, and its relation to dental disease are under way.

SUMMARY

Hyaluronidase activity has been demonstrated in human saliva.

The relative activity of hyaluronidase has been shown in a random selection of 64 subjects.

The author wishes to express his appreciation to Dr. Z. Hadidian, Department of Pharmacology, Tufts College Medical School, and to Drs. R. S. Manly and H. A. Zander, Tufts College Dental School, for the valuable suggestions and assistance they have given.

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SOME MECHANICAL CHARACTERISTICS OF AMALGAMATORS

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IT HAS been well established that the characteristics of dental amalgams are subject to wide variation as the result of changes in formulae, manufacturing methods, and trituration and packing procedures.

Trituration and packing procedures are under the control of the operator. In any given alloy we expect to find that undertrituration will produce low crushing strength, high flow and higher than normal expansion in finished amalgams. The proper amount of work applied during amalgamation will provide acceptable expansion (3-13 microns per cm.) and less than 4 per cent flow in all alloys "Certified" to the Research Council of the American Dental Association. Overworking will increase flow values and will decrease expansion if it does not actually cause shrinkage in the final fillings.

The susceptibility of alloys to such changes may vary widely from one product to another, some being markedly stable compared with others, but variations of 15 per cent in the mercury-alloy ratio have been shown by Phillips and Boyd[1] to have marked effects on the properties of amalgams and presumably variations of 10 per cent may be significant.

In recent months there appears to have been an increasing interest in mechanical amalgamators. Individuals are undoubtedly led to consider these devices because of their desire to do this type of work as easily and conveniently as possible, and in many cases it seems that claims for speed and convenience have been so alluring the purchasers have neglected to question the effects of the instruments on the properties of finished restorations.

Since the effects of variations in working have been so well established, and further, considering the increasing use of mechanical amalgamators, it appears that every factor governing the use of the instruments should be considered.

Mechanical devices to facilitate the amalgamation of alloy particles with mercury for use in dental restorations have been offered the dental profession for many years. The saving of time and the increased convenience have been emphasized. Mechanical amalgamators seldom have been accompanied by adequate directions from either the manufacturers of the instrument or of the alloy, and the user has been left to his own devices in working out a satisfactory method for its use. Under these circumstances we find that some consideration has been given to the effects of mechanical trituration on different types of alloys, but little if any to the mechanical characteristics of the instrument which might vary the amounts of work done and so affect the characteristics of the finished amalgams.

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It is well established that a variety of factors may definitely influence the final properties of amalgams. These are factors under the control of the manufacturer:

1. The formula selected.
2. The preliminary annealing (if any) of the cast bars of alloy.
3. The gradation of particle size and shape.
4. The final annealing and cleaning of the particles.

The manufacturer is expected to supply a product to meet the requirements of the American Dental Association Specification No. 1 for Dental Amalgam Alloy, and therefore must select a formula and control his methods carefully to give an alloy of acceptable quality. He must also supply adequate directions for the use of his product. These directions must cover those factors under the control of the operator which may affect the quality of the finished restoration, namely:

5. The proportion of alloy to mercury.
6. Mixing procedure involving:
   a. Mixing time.
   b. Rate of pestle rotation.
   c. Pressure on pestle, all assuming that the size of the mortar is correct for the size of the mix, the fit of the pestle to the mortar is close, and the surfaces of both mortar and pestle are clean and etched to permit efficient mixing.
   d. The time elapsing between mixing and packing.
   e. The method of expressing excess mercury before packing.
   f. Packing procedures which include such factors as the size and shape of instrument surfaces, and the forces applied to them.

In packing amalgams the amount of mercury expressed affects both flow and expansion. Low packing pressures or prolonged working times will give higher than normal expansions and flow, and low crushing strength. High pressures will lower expansion and flow.

Sedimentation or separation of alloy particles into portions having different particle sizes will also affect final results, the finer sizes amalgamating more rapidly and having lower expansion and higher flow.[2] Mechanical amalgamators are recommended to facilitate the mixing of alloys with mercury, and to save time by reducing the mixing time to a matter of a few seconds. Granting that the mixing of amalgams is a critical process, it appears that any speedup of the correct mixing process must be carefully evaluated and controlled if undesirable effects are to be avoided. Also, the amount of time required to mix different alloys currently used is quite varied, as shown by the "normal mixing time" on a particular amalgamator (Rose) reported by the authors on five dental alloys as 8, 13, 15, 20, and 20 seconds, respectively.[3]

This study of the mechanical characteristics of amalgamators was initiated with the object of estimating possible damaging effects of mechanical amalgamation upon the properties of the finished amalgam restorations.

A group of mechanical devices which were operated through the conventional handpiece was given no more than casual consideration. This type of equipment can be passed over quickly by observing that it is operated without timing controls, and that its speed depends upon the speed of the motor used and the belt slippage. The variables appear so great that little hope can be held out for controlled amalgamation. Little information could be gained by detailed study of them. Their effects on either amalgams or handpieces are uncontrolled and are almost certainly harmful to both.

The group of amalgamators most popular are those driven by small motors which are part of the unit itself. Three types of these were studied to determine possible effects of their mechanical characteristics upon amalgams made in them. The amalgamators studied were:

A. *The Wig L Bug.*—This has a synchronous motor and a timer which can be set for a given time interval and which stops the machine at the expiration of that interval, once the starting switch is thrown. The alloy and mercury are shaken with a reciprocating motion in either a metal or plastic capsule with or without a small metal pestle.

B. *The Rose Amalgamator.*—This machine is driven by a small motor and amalgamates the alloy and mercury by shaking them together in a cylindrical plastic container (pillbox) with a rotary action. The machine is started by turning a switch against an adjustable stop. This starts the motor which operates until a switch mounted on the shaft of a reducing gear breaks the circuit.

C. *The Flossy Amalgamator.*—A small motor drives this machine which uses a reciprocating shaking action to obtain amalgamation. Timing is controlled by a lever which operates a timing device and also starts the motor.

Inasmuch as any factor which changes the amount of work done on an alloy-mercury mixture during amalgamation will affect the finished properties, studies were made of these amalgamators to determine: (1) Accuracy of timing; (2) effects of voltage variation; and (3) effects of size of load.

The equipment used to control and measure variables included: (1) A small variable transformer which made it possible to operate the instruments on all voltages within the 100-120 volt ranges encountered in service; (2) a high grade volt meter to indicate voltages supplied; (3) stop watches and an electrical laboratory timer; and (4) a General Electric Company Strobotac—a stroboscopic instrument for determining the motor speeds to an accuracy approximating 10 r.p.m.

Line voltages in dental offices vary with location, total line load, and external light conditions, and variations from 100 to 120 volts may be encountered on 110 volt lines.

Speeds of the instruments were determined when operating at various voltages and under various load conditions and the data secured are shown in Table I.

The Wig L Bug, having a synchronous motor, operates at almost constant speed. The power of its motor is limited and this, together with the space limitations of its capsules, makes it inadvisable to make mixes larger than 12

TABLE I
SPEED VARIATIONS OF AMALGAMATORS

VOLTAGE (60 CYCLE A.C.)	LOAD (GR.)	ROSE AMALGAMATOR (R.P.M.)	FLOSSY* AMALGAMATOR (R.P.M.)	WIG L BUG	
				WITH PESTLE (R.P.M.)	WITHOUT PESTLE (R.P.M.)
100	empty	1490	1925	1380	1470
	6	1465	1780	1300	1440
	12	1365	1700	1290	1420
	18	1310	1540	1330	1370
105	empty	1500	1990	1420	1510
	6	1480	1890	1370	1490
	12	1390	1780	1345	1470
	18	1350	1670	1365	1430
110	empty	1510	2040	1460	1550
	6	1500	1960	1430	1520
	12	1410	1860	1400	1500
	18	1380	1790	1400	1470
115	empty	1540	2040	1490	1565
	6	1505	2020	1470	1550
	12	1450	1930	1440	1525
	18	1400	1900	1440	1500
120	empty	1565	2060	1510	1575
	6	1520	2090	1490	1560
	12	1460	1990	1465	1550
	18	1420	1950	1470	1515

*The manufacturer advertises a speed of 2500 r.p.m. When first received this machine operated at approximately that speed, but after comparatively little use the speed dropped to the levels shown.

grains of alloy plus the required amounts of mercury. The Rose instrument after months of regular use operates at the same speed as when received. Its capsules are also too small to handle larger than 12 grain mixes satisfactorily. A new Flossy instrument operated at 2500 r.p.m. on 110 volts when received, but in a comparatively short time and with relatively little use it changed enough that its operating speed dropped to 2000 r.p.m. (20 per cent).

I. *Timing.*—The timer of the Wig L Bug, being set independently, will give as great a uniformity in time intervals as the operator's uniformity in setting it. Inasmuch as the time intervals used are short, care in setting the timer is an important factor in the use of the machine.

The timing of the Rose Mixer is mechanical and if the starting switch is turned promptly to the stop and released, the machine will operate for a fixed number of revolutions and stop. It should be noted here that though the speed of the motor may be changed by changes in load or voltage, the total number of revolutions will be unchanged, and if the speed variations are not abnormal, the amalgamation produced will be reasonably uniform.

The timer of the Flossy machine has the starting switch incorporated into it. The scale indicating time intervals is small and individuals vary in the time taken to get the desired setting; results were erratic enough to indicate undesirable irregularity of performance. Four operators were used in timing tests on this machine, setting certain time intervals which were checked against actual time of operation.

II. *Effects of Voltage and Loading.*—The three machines were studied when operating on controlled voltages within normal operating limits and with different loadings. The loads used were 6, 12, and 18 grains of alloy, and alloy to mercury proportions were all 5:7. Speeds were checked with a General Electric Strobotac.

SUMMARY

1. Mechanical mixers operated on handpieces, being so variable in performance, are not recommended for use if an operator hopes to produce high grade restorations consistently.

2. The Flossy Amalgamator showed poor timing control and variations in motor speed.

3. The Wig L Bug was only slightly affected by voltage variations, but practical experience with it indicated that it should not be used for mixes larger than 12 grains of alloy.

4. The Rose Mixer has the same load limit as the Wig L Bug. The timing device is quite uniform for loads of 12 grains.

5. It is suggested that the best way to use an amalgamator in the absence of specific directions for the use of the alloy at hand would be to make several mixes by hand and then make several mixes under varying conditions with the machine. Then by comparing the satisfactory mix obtained by hand with the mechanical mixes, the time and conditions can be fairly well established.

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AN IMPROVED METHOD FOR THE PREPARATION OF "SERIAL" SECTIONS OF UNDECALCIFIED DENTAL TISSUES

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SINCE our previous report[1] various improvements on the existing methods of sectioning undecalcified dental tissues have come to our knowledge. Lefkowitz[2] used a rapidly revolving large diameter phosphobronze disc, the circumference of which was charged with diamond powder. The width of the cuts produced by his apparatus is not given, but it cannot have been less than 0.150 mm., this being the thickness of the disc. As the thickness of the sections is stated to vary from 0.250 to 0.400 mm., this method can at best have yielded an average of 2 sections per mm. Sognnaes[3] cuts a number of sections simultaneously with a set of circular steel saws each 0.250 mm. thick. Only one 0.750 mm. section is produced per mm. But, as in the case of Lefkowitz' apparatus, comparatively large sections can be obtained (e.g., complete longitudinal sections of human molars). His introduction of methyl methacrylate as an embedding medium constitutes an important improvement of the technic. In a very simple arrangement Atkinson[4, 5] employed bonded carborundum discs from 0.8 to 0.125 mm. in thickness, but it is not stated how many sections per mm. can be obtained. In this case too, large sections can apparently be made.

In 1946 we[1] reported on a method producing small sections at the rate of 5 sections per 2 mm. The number of sections per mm. and the size of the sections seemed capable of improvement. A large and very thin circular saw blade is capable of cutting large sections and at the same time causing small losses of material as narrow saw cuts can be made with it. The unstability of its cutting edge calls for an allowance in the distance of the successive saw cuts (i.e., in the thickness of the slabs) which precludes an approach to serial sectioning.* On the other hand, as in our 1946 machine, the unwavering edge of a narrow and thick saw blade enables very thin sections to be cut, but there is a regrettable loss of material and moreover only small blocks of tissue can be cut.

In general, a thin free saw blade can be given a firmness comparable to that of a narrow thick blade by subjecting it to a tension as is done in the ordinary bow saw. Our first apparatus built on this principle was a miniature reciprocating saw. The length of the saw blade (a narrow strip of phosphobronze 2 mm. in width and 0.120 mm. thick) was 3 cm. and the stroke 2 cm. It was driven at the rate of 150 strokes per minute. Diamond powder applied to the edge of the saw blade acted as a cutting agent. Owing to the relatively short stroke and the slowness of action only small pieces of dental tissue could be sectioned by this machine, but not less than four 0.075 mm. sections per mm. could be obtained. The saw cuts were 0.175 mm. in width.

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*Though the centrifugal force tends to stabilize conventional circular saw discs, according to our experience its effect is too weak for our purpose at the speeds used (up to 10.000 r.p.m., diameter 90 mm.).

As far as we can ascertain this gave a closer approach to serial sectioning than had hitherto been achieved, so we endeavored to develop a circular saw, built on the same principle, which would allow cutting of at least as many sections per mm. but at a much greater speed and from larger blocks of tissue.

Fig. 1 gives a general view of an apparatus complying with the above specifications. The saw blade (*a*) is provided with a large central hole. The edge of this hole is the cutting edge of the saw. The outer circumference of the saw blade is fastened to the rim of a drum (*b*). The hollow portion of the drum accommodates the parts of the object and the object carrier projecting beyond the plane of the saw blade. The drum (*b*) it attached to the shaft (*d*) which in turn is supported by two bearings (*h* and *j*) at either end of the tube (*e*). Drum and shaft are rotated by an electric motor by means of the belt (*c*). As a rule the object is embedded in methyl methacrylate. The block of plastic is fastened to the object holder (*f*) by means of wax. It is gently pressed against the cutting edge by a spring (not shown in Fig. 1). Two strips of sheet metal (g_1 and g_2) act as a hinge without any play, the imaginary axis of which should be exactly at right angles to the plane of the saw blade.

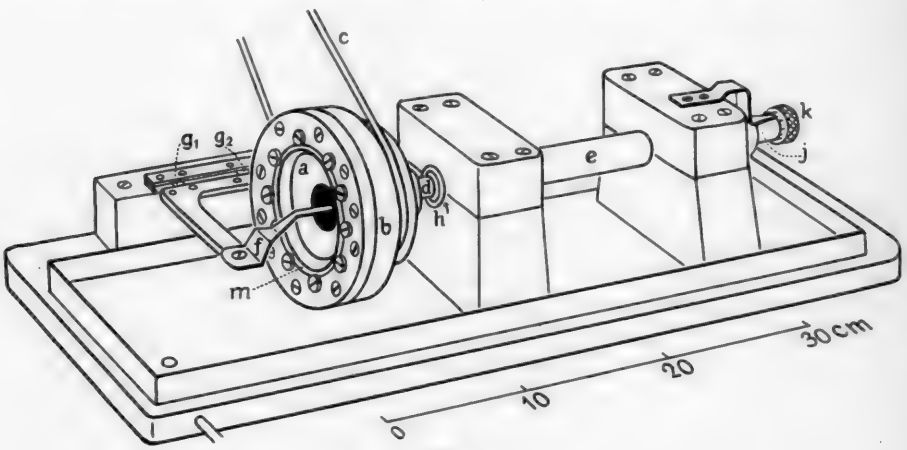

Fig. 1.

The shifting of the saw, enabling a row of saw cuts to be cut successively, is brought about in the following way: bearing *h* allows a rotating as well as a sliding movement of the shaft, whereas any axial displacement of the shaft relative to bearing *j* is prevented. Now bearing *j* can be screwed to and fro in the tube (*e*) the axial movement resulting therefrom being readable in tenths of mm. Thus one can, by turning the knurled end (*k*) of bearing *j*, effect a known displacement of the shaft and consequently of the saw.

The manner in which the saw blade is fastened and stretched can be seen in the Figs. 2 and 3. In these illustrations the drum, designated *b* in Fig. 1, is shown to consist of the drum proper (b_1) and the ring (b_2). The outer zone of the saw blade is clamped between these parts, which are pressed firmly

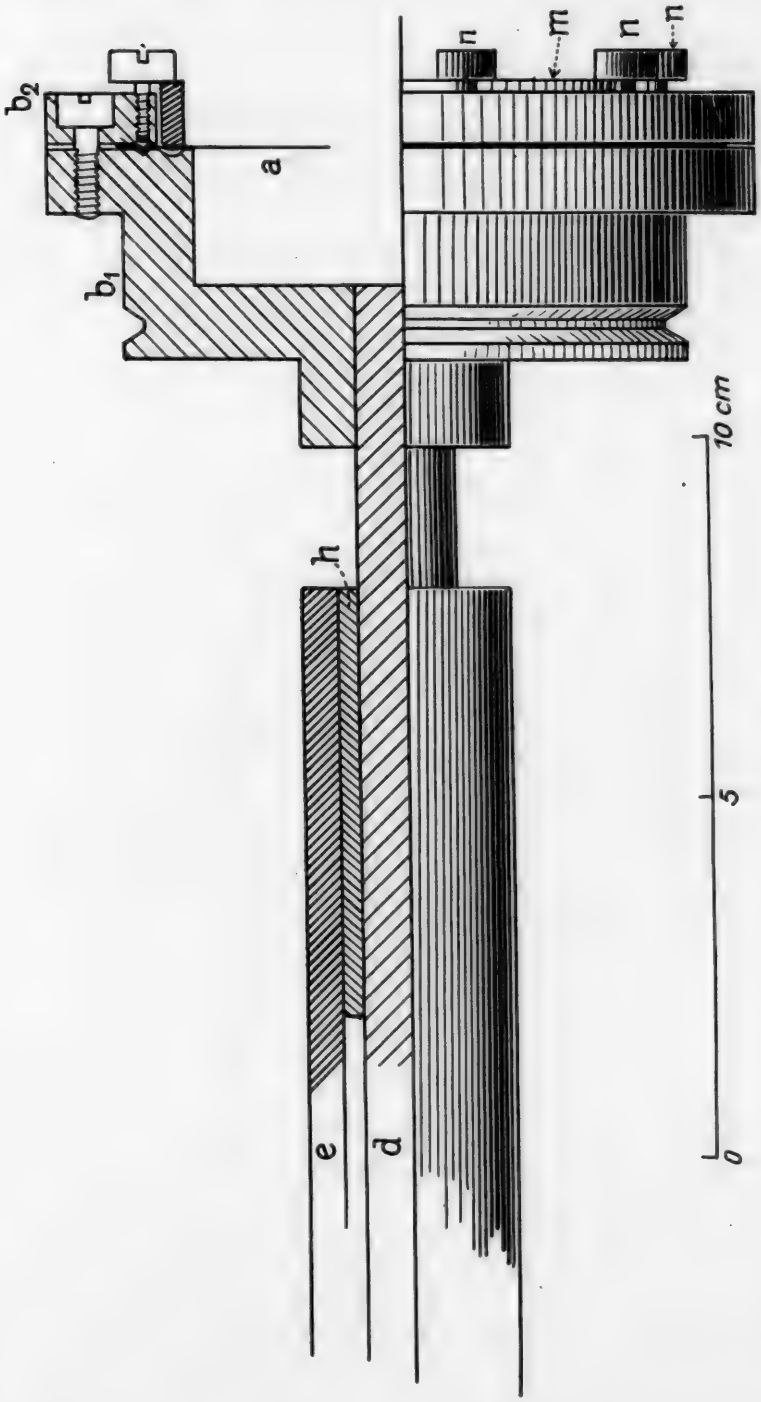

FIG. 2.

against each other by means of 10 studs (l). A sharp circular ridge on the ring (b_2) fitting into a groove in the drum serves to prevent any movement of the periphery of the saw blade.

Stretching of the saw blade is brought about by means of the ring m. This part is pressed against the saw blade by the heads of ten studs (n), causing a circular deflection of the saw blade into the groove o. This device allows the saw blade to be very effectively stretched. If the ridge (p) is in one plane at right angles to the shaft (d) the whole of the blade, from p down to the cutting edge, will also be in one plane. It has been found advisable to start by fastening and stretching a sheave of phosphobronze. The hole, turned out afterwards with a sharp narrow chisel, automatically will be perfectly centered.

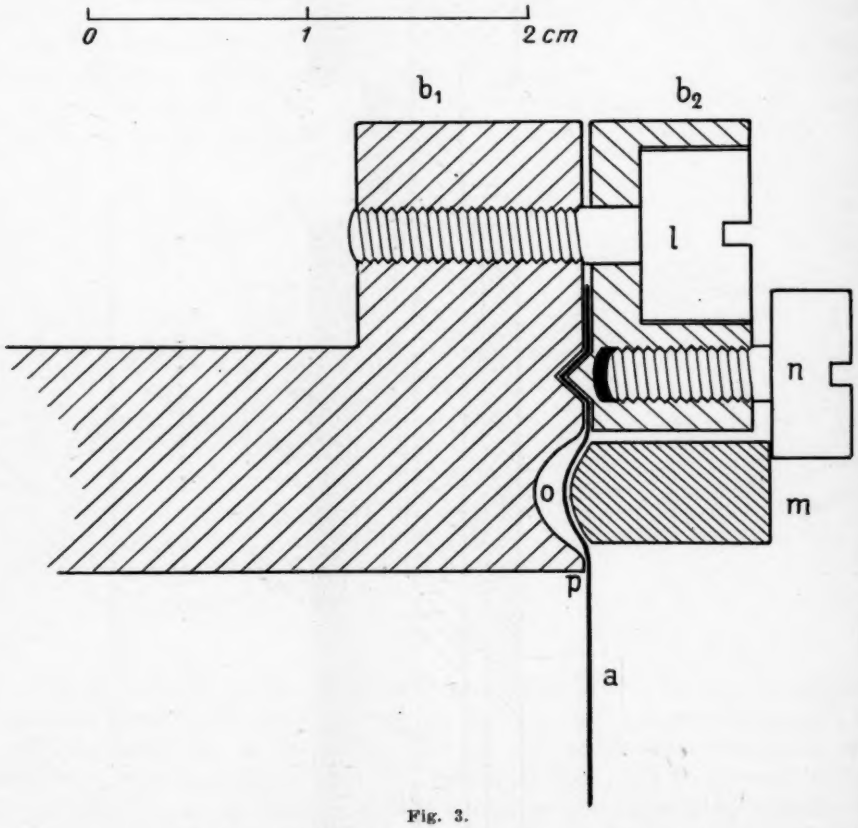

Fig. 3.

In order to charge the cutting edge with diamond powder one prepares a suspension of one carat of diamond powder in 8 drops of olive oil, this suspension being prepared at least 24 hours prior to being used. A small quantity of the suspension is applied to the surface of a hardened steel roller, which is allowed afterwards to run against the cutting edge of the slowly rotating saw for about

10 minutes. One should learn by experience the appropriate pressure and speed for the sharpening process.

Correct sharpening results in the cutting edge becoming broadened. The effect of this is shown diagrammatically in Fig. 4. Though the saw cut is apparently unduly widened, we feel that this disadvantage is more than compensated for by the fact that in case of proper adjustment of the hinge of the object carrier only the cutting edge of the saw need come into contact with the block of tissue.

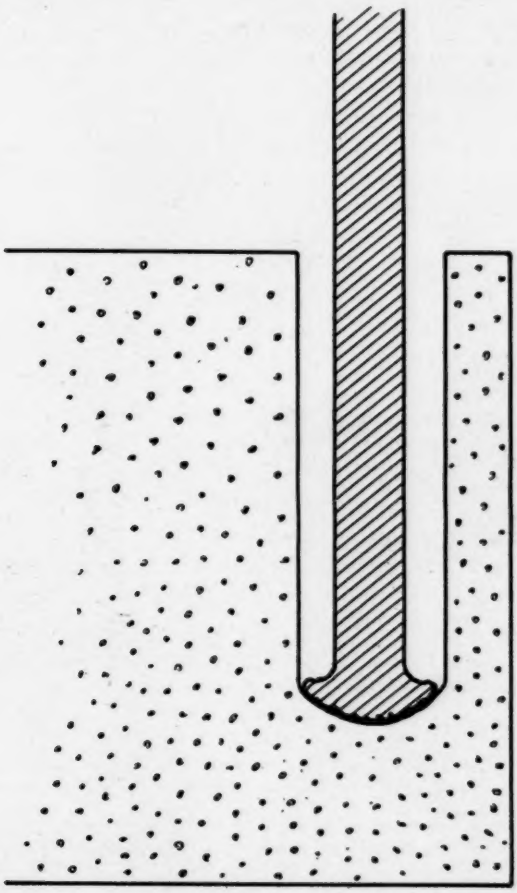

Fig. 4.

As a result undue pressure of the saw blade on the section need not be feared.

The saw will only work if the cutting edge is kept wet. Water is commonly used for this purpose, but under certain circumstances (e.g., in experiments with water-soluble dyes or if one tries to prepare sections from frozen pieces of tissue) its use may be prohibited. Accordingly, we have tried numerous

organic liquids, but water proved to be preferable by far with regard to the speed of the cutting. (For the sake of clearness neither the water supply nor the spatterguard is shown in Fig. 1; a simple electric device stopping the machine as soon as a cut is completed has been omitted for the same reason.)

The saw blades we used were 0.060 mm. thick. With these 0.150 mm. saw cuts were obtained, the sections being 0.100 mm. thick. Consequently 4 intact sections could be made per mm. of tissue. The maximal area of the sections produced, mainly depends on the standard of accuracy maintained while the machine was under construction. We have been able to cut four sections per mm. from blocks of tissue measuring up to 1 × 1 cm. in cross section.

As the cutting proceeds the object moves along the saw blade and it is of paramount importance that this movement be exactly parallel to the plane of the blade. Otherwise, the block of tissue or the sections will eventually come into contact with the saw blade at some distance from the cutting edge, which is undesirable as damage to the section will be the result. This consideration calls for a robust object carrier. As has already been stressed before,[1] however, the object holder should on the other hand be as light as possible to ensure smooth sawing at high speeds. Eventually we adopted the construction of an aluminum object holder hinged in strips, which constitutes a compromise of these conflicting demands.

At 5,000 r.p.m. of the cutting wheel the sections take from a couple of minutes to a quarter of an hour to complete, depending on the size of the block and on the embedding technic, methyl methacrylate being cut decidedly more slowly than dentin or enamel.

SUMMARY

An apparatus is described with which four 1 × 1 cm. sections approximately 0.100 mm. thick per mm. of undecalcified dental tissues can be cut. This is achieved by using a mechanically stretched, very thin circular saw blade, the border of a central hole in the blade serving as cutting edge.

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